Accepted 4/10/87

# THE EFFECT OF CATIONS ON ZOOSPORES OF THE FUNGUS PHYTOPHTHORA CINNAMOMI

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Running Title: Effect of ions on *P. cinnamomi* zoospores

Subject Category: Physiology and Growth.

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Index phrases:

1. *Phytophthora cinnamomi*
2. Zoospores, encystment and germination of
3. Zoospores, effect of cations on
4. Encystment of *Phytophthora* zoospores
5. Germination of *Phytophthora* zoospores

SUMMARY

Swimming zoospores of the fungus Phytophthora cinnamomi were exposed to a range of ions and found to be much more sensitive to cations than anions. One cation, $Ca^{2+}$, induced zoospores to encyst and subsequently germinate, but most cations induced encystment only and were toxic at higher concentrations. In general the sensitivity of zoospores to a cation increased with its charge density. At. 0.3 μM, $La^{3+}$ reduced the viability of a zoospore population to 50%, while $Fe^{3+}$ (20 μM) and $Mn^{2+}$ (50 μM) induced encystment with only a slight decrease in viability. The other divalent and monovalent cations tested ($Mg^{2+}$, $Ca^{2+}$, $Ba^{2+}$, $Li^{+}$, $Na^{+}$, $K^{+}$, $Cs^{+}$, $NH_4^{+}$) were effective in inducing encystment or reducing viability at higher concentrations. Each ion showed a distinctive concentration-response curve. Only $F^{-}$ and $CH_3COO^{-}$ among the anions tested ($Cl^{-}$, $NO_3^{-}$, $F^{-}$, $H_2PO_4^{2-}$, $SO_4^{2-}$, $CH_3COO^{-}$) had any effect on zoospores, and at 20 mM (pH 6.0) they reduced viability.

The cysts (cystospores) of the fungus were generally less sensitive to cation effects than swimming zoospores, and only $Cs^{+}$ and $K^{+}$ (50 mM) reduced viability to the same extent in each population.

Both zoospores and cysts of this fungus had a broad tolerance to pH and temperature and cysts were more resistant to low temperatures than motile zoospores.

## INTRODUCTION

The biflagellate zoospores of the Oomycetes go through the process of cyst (cystospore) formation and cyst germination after their release from sporangia and before the formation of a new mycelial stage. The changes in ultrastructure which take place during this transformation have been described for a variety of species and genera (for a list of references see Lunney & Bland, 1976). The details of the ultrastructural changes during the process of cyst formation and germination have been particularly well documented in the genus Phytophthora (Hemmes & Hohl, 1971; Tokunaga & Bartnicki-Garcia, 1971; Bimpong & Hickman, 1975; Sing & Bartnicki-Garcia 1975). However, there is less information on the mechanisms which trigger these developmental changes.

The external ionic environment is known to affect zoospore differentiation in both the monoflagellate Blastocladiella emersonii and the biflagellate Aphanomyces astaci (Soll & Sonneborn, 1972; Svenson and Unestam, 1975). There are indications that Phytophthora zoospores are similarly affected since the motility of P. palmivora (Butl.) Butl. was reduced by $Ca^{2+}$ and $Mg^{2+}$ salts (Bimpong & Clerk, 1970). In addition P. palmivora zoospores exhibited negative chemotaxy in response to a range of cations (Cameron & Carlile, 1980). In the case of the plant pathogen P. cinnamomi Rands, zoospores were repelled by inorganic cations (Allen & Harvey, 1974), and there is evidence that the disease pattern was influenced by soil cation levels (Broadbent & Baker, 1974; Halsall & Forrester, 1977; Boughton et al., 1978).

In this paper we show that in P. cinnamomi the transformation of swimming zoospores to germinating cysts is affected by the external concentrations of a variety of cations. The results suggest that specific ion effects are involved rather than changes in osmotic pressure. The significance of the results in relation to the potential behaviour of the parasite in soil and its capacity to act as a pathogen is discussed.

METHODS

The isolate of P. cinnamomi (A-2 compatibility type) was obtained from the Brisbane Ranges, Victoria, by Dr. G. Weste and designated by her as isolate PC110 (Commonwealth Mycological Institute Collection IMI 252489). Zoosporangia were induced and zoospores released from axenic cultures as described in a previous publication (Byrt & Grant, 1979). After release the zoospores ($10^4$ - $10^5$ cells $ml^{-1}$) were held in tris-succinate buffer pH 6.5 (1 mM) at 16±1°C and used within 2 h. All glassware with which zoospores came in contact was prewashed with 2 M HCl overnight prior to use. All operations in which zoospores were transferred or mixed required care to minimise mechanical or pressure shock. Zoospores were exposed to the various ions in plastic tissue culture trays (Linbro, Connecticut, U.S.A.) for up to 120 min. Unless stated the pH was 6.5. When cyst populations were to be tested, they were induced from swimming zoospores by 2 min vigorous shaking (Tokunaga & Bartnicki-Garcia, 1971). Cells were examined directly or after fixation with 2% glutaraldehyde, pH 6. The numbers of cells and their developmental stage were counted using an inverted microscope with a calibrated grid in the ocular. A volume of 1.5 or 2.0 ml per chamber was used, and all incubations carried out at 19±2°C. After fixation, cells were stable for at least a week at room temperature, but counting was always completed within 4 days of an experiment.

When experimental procedure required that cells be transferred from one container to another, cells at different stages of development were not recovered with equal efficiency. Thus the protocol was designed so that the entire experimental procedure could be carried out in one vessel. All experiments contained controls in which zoospores were held in 1 mM buffer during the period of treatment. All results are reported as percentages obtained from counts of 100-200 cells. The percentage of encysted cells in the controls varied from one experiment to another but was usually in

the region of 20%. All allocations of treatments were made on the basis of random number tables (Fisher & Yates, 1963) and the samples counted blind.

It was possible to estimate the percentage of damaged cells by a direct count. However, the distinction between a damaged and an undamaged cell was not always clear cut. Another method of assessing the viability of cells was to test their ability to germinate under optimal conditions. The addition of a mixture of V8 broth (5%) and $Ca^{2+}$ (4 mM) was suitable for this purpose. The percentage of germinated cells was then used as an estimate of the viable cells in a population. All assays for viability were carried out at 22±2°C for 180 min, before the cells were killed by glutaraldehyde addition. Viability assays were carried out in the presence of the ionic species under test. It was shown in preliminary experiments that if the ions under test were added to swimming zoospores at the same time as the $Ca^{2+}$/V8 medium only $Cs^{+}$ had any effect on germination. In 50 mM $Cs^{+}$, germination was reduced from 98±1% to 92±2%.

## RESULTS

Three stages of zoospore development were distinguished in this work.

1. Motile zoospores: After fixation, these were ovoid to elliptical in longitudinal view, and elliptical with a well-defined groove when viewed end on. No cell wall was visible and the cells had a clear or translucent appearance. Flagella were visible when the cells were viewed under phase contrast or Nomarski interference microscopy. The cells appeared refractile under bright field microscopy.

2. Cysts or Cystospores: These were round, with a clearly defined outline.

3. Germinated Cysts: These cells had clearly visible germ tubes. However, some treatments gave rise to cyst papillae (Palzer, 1976; Ho & Zentmyer, 1977).

The cyst papillum appeared as a clearly defined swelling or protuberance on the surface of the cyst.

In addition, damaged cells were present after certain treatments. These were sometimes swollen, and lacked the clear outline of undamaged cells. Some treatments also induced a high proportion of lysed cells, and cell fragments were visible. The extent of lysis was measured by the difference between the initial and the final cell concentration.

The Effect of Temperature

Both viability and motility of zoospores were affected by temperature. The highest proportion of motile cells was maintained between 10° and 20°C although even in this temperature range a high proportion of cells encysted during the 2 h incubation period (Fig. 1). Below 10° and above 25° both the number of motile cells and the number of viable cells decreased rapidly. Temperature also affected the behaviour of the cells in more subtle ways. For example, above 25°, the zoospores were much more liable to encyst as a result of mechanical shock. Cyst populations were similar to zoospores in their response to temperatures above 25°. By contrast, cysts were much more resistant than zoospores to temperatures below 10°C (Fig. 1).

The Effect of pH

Zoospores remained motile over the pH range 4.5 to 8.5 and viability did not vary over this same range. At the extremes of pH, the number of motile zoospores fell rapidly (Fig. 2). As the $H^+$ concentration increased above 10 µM (pH 5), an increasing proportion of zoospores was immobilized. They rounded up, but only a small proportion (less than 30% at pH 4.1) formed cyst walls resistant to 1 M KOH. After exposure to pH 4.1 for 1 min the immobilized cells could not form alkali-resistant walls on shaking, nor when returned to pH 5.8 and incubated for 30 min. The response of cysts in viability tests at the extremes of pH was indistinguishable from that of zoospores.

Effect of Cations

Zoospores of P. cinnamomi were affected by both the species of cation and its concentration. The effects observed ranged from transitory changes in swimming pattern to increase in the rate of encystment and/or decreases in the viability of cells. Here we report only the quantifiable effects on the transitions from zoospore to cyst and cyst to germinating cyst, as well as loss of viability. The rates at which ions induced changes in zoospores varied widely. Some ions, such as $K^+$ and $Ca^{2+}$ acted rapidly. Exposure to $K^+$ (10 mM) immobilized 80% of the zoospore population in 7 min and $Ca^{2+}$ (25 mM) had the same effect after 20 min. On the other hand, $Mg^{2+}$ (25 mM) acted much more slowly, requiring 90 min to immobilize 80% of the zoospores. $Na^+$ had a partial effect only, as exposure to $Na^+$ (25 mM) for 15 min immobilized 50% of the zoospore population but there was then no further immobilization in the next 45 min.

The effect of various concentrations of $Li^+$, $Na^+$, $K^+$, $Cs^+$ and $NH_4^+$ on immobilization of the swimming zoospores is shown in Figs. 3 and 4. There were great differences in the response to the different ions, with $Li^+$ inducing no encystment up to 50 mM while 5 mM $K^+$ induced essentially complete encystment. The order of effectiveness in the induction of encystment was $K^+ > NH_4^+ > Na^+ > Cs^+ > Li^+$. However, the effect on viability was rather different (Fig. 5). Exposure to $K^+$ reduced viability to 60% at 14 mM but increase in $K^+$ concentration above this level did not decrease viability further. Exposure to $Na^+$, $NH_4^+$, $Li^+$ and $Cs^+$ all resulted in decreased viability as concentrations increased.

Both $Cs^+$ and $NH_4^+$ treatments resulted in a high proportion of swollen and obviously damaged cells. The $Li^+$ treatment produced cells which had the appearance of motile zoospores but which moved only sluggishly, and these cells did not encyst or germinate on addition of $Ca^{2+}$/V8 broth. The order of effectiveness of Group I cations in causing cell damage was $Cs^+ > Li^+ > K^+ > Na^+$.

The damage caused by $NH_4^+$ relative to the other monovalent cations tested was very dependent upon concentration. Below 10 mM $NH_4^+$ had little effect on either motility or viability. However, at 50 mM it was second only to $K^+$ in causing immobilization and to $Cs^+$ in the reduction of viability of zoospore populations.

Cells incubated in solutions of mannitol or sorbitol at concentrations up to 100 mM did not show significant increases in the rates of encystment or decrease in viability. This, together with the differences in the effects of individual ions, makes it very unlikely that changes in osmotic pressure have a large effect on the processes observed. Even NaCl, the salt least effective in inducing encystment was more effective than mannitol or sorbitol.

The effect of four divalent cations of $GpII_A$ is shown in Fig. 6. All the ions in this series promoted encystment, with $Sr^{2+}$ being the most effective. $Mg^{2+}$, the least effective, required 90 min exposure before detectable effects could be observed. At this time cells were rounded and often swollen with some retaining flagellar activity. This resulted in such cells exhibiting a slow rotary motion. At a concentration of 10 mM the order of effectiveness in inducing encystment was $Sr^{2+} > Ca^{2+} > Ba^{2+}, Mg^{2+}$. Both $Ca^{2+}$ and $Sr^{2+}$ induced encystment without any cell damage, and in $Ca^{2+}$ encystment was followed immediately by germination. The germination rate was higher in $Ca(NO_3)_2$ than in $CaCl_2$, and this was one instance where the anionic species influenced the zoospore response. Germination did not immediately follow encystment after the addition of $Sr^{2+}$, unless nutrient was added.

The trivalent cation $La^{3+}$ has a similar ionic radius to $Ca^{2+}$ and has been shown to act in a similar fashion in a number of biological systems although at lower concentrations (Takata et al., 1967). When $La^{3+}$ was added to zoospore suspensions it was found that cells were immobilized at 0.1-1 μM

almost immediately (Fig. 8, Table 1). Cells which had been treated with $La^{3+}$ were not viable, and at $La^{3+}$ concentrations above 1 μM there was complete lysis of the cell population. Therefore $La^{3+}$ does not appear to act as an analogue of $Ca^{2+}$ in this system, and mimics $Ca^{2+}$ only to the extent of causing immediate immobilization.

$Fe^{3+}$ and $Mn^{2+}$ ions both induced encystment of the entire populations of cells, $Fe^{3+}$ at 15 μM and $Mn^{2+}$ at 500 μM. However, cells which encysted in the presence of these ions were completely viable, and thus $Fe^{3+}$ and $Mn^{2+}$ resembled $Ca^{2+}$ and $Sr^{2+}$ rather than $La^{3+}$ in their effect (Table 1). The trivalent cation $Al^{3+}$ had no effect on either viability or encystment at the highest concentration tested (30 μM, pH 5.5).

In short, P. cinnamomi zoospores were found to be sensitive to all the cations tested. Different cations caused responses which differed both qualitatively and quantitatively and these are summarized in Table 2. The trivalent cations, $Fe^{3+}$ and $La^{3+}$, were effective at concentrations several orders of magnitude lower than those required for the mono- and divalent cations, but $Al^{3+}$ was without effect.

## Effect of Anions

In all of the experiments described in the foregoing the chlorides were used, except in the case of $La^{3+}$, in which the $NO_3^-$ was used. There was no difference between the $Cl^-$, $NO_3^-$, $SO_4^{2-}$ and $PO_4^{3-}$ when $Na^+$ was the common cation (Table 3). Where there was an increase in the percentage of encystment it could be shown to be due to the variation in $Na^+$ concentration. Acetate, and to a lesser extent fluoride, reduced zoospore motility and viability.

## Effect of Cations on Cysts

When cysts which had been induced to form by shaking were incubated in the presence of the various cations it was found that they were less

sensitive than the swimming zoospores (Table 4). Only $Cs^+$ reduced the viability greatly, to 3% at 50 mM. Both $K^+$ and $NH_4^+$ also reduced viability but none of the other ions tested had significant effects ($P = 0.05$).

## DISCUSSION

Our results showing the sensitivity of P. cinnamomi zoospores to cations resemble those described by Svensson & Unestam (1975) for the biflagellate oomycete, A. astaci, zoospores, although it is difficult to make direct comparisons as those authors did not distinguish quantitatively between encystment and germination. They found that LiCl and CsCl were toxic, that zoospores were more sensitive to KCl than to NaCl, and that germination rates were highest in the presence of $CaCl_2$. They concluded that the initiation of germination was partly due to an osmotic effect on the zoospores as mannitol and mannose were also effective. However, in their work, as in ours, mannitol was less effective than NaCl, and much less effective than KCl or $CaCl_2$, and it seems more likely specific ion effects were involved.

Soll & Sonneborn (1972) also concluded that ion effects on B. emersonii zoospores were not due to osmotic shock, as sucrose had no effect in their system. In many respects, however, the ion selectivity patterns observed with the monoflagellates differed significantly from those for biflagellates. $K^+$, $Na^+$, $Cs^+$ and $Rb^+$ salts were all found to elicit encystment and germination of B. emersonii zoospores, although $Li^+$ was found to be toxic. Conversely $Ca^{2+}$ and $Mg^{2+}$ were less effective and germ tubes formed in response to $Ca^{2+}$ appeared to be defective, and $NH_4^+$ ions had little effect.

Soll and Sonneborn performed their experiments in the presence of low levels of other ions, and low concentrations of ions are thought to be essential for motility of both monoflatellate and biflagellate zoospores

(Cameron & Carlile, 1980). In our experiments no attempt was made to exclude low concentrations of ions either carried over as leachate from the washed mycelium or from the glass containers in which mycelium and zoospores were held. Therefore, we would expect that low concentrations of ions, in the μmolar region, would be present at all times. Such low concentrations are apparently sufficient to maintain the motility and viability of P. cinnamomi zoospores. At the higher ion concentrations tested in our work, the effect of $Ca^{2+}$ was unique. Although both $Sr^{2+}$ and $K^{+}$ were more effective in inducing encystment, only $Ca^{2+}$ triggered encystment followed by germination. Although our experiments do not point to any mechanism, they suggest that $Ca^{2+}$ may well play a specific role in nature in the developmental cycle of this fungus.

It is well established that cations interact with biological membranes at concentrations comparable with those used in the present studies (Williams, 1972). The ability to distinguish between closely similar ions and molecules is a basic function of cell membranes. A similar selectivity is also shown in a range of non-biological systems. It has been shown that the relative order of activity of the alkali-cations and cations of higher valencies with many biological and non-biological systems is restricted to a few sequences. These include the Hoffmeister lyotropic series and are related to the field strength of the ions under different conditions (Diamond & Wright, 1969). Thus it may be predicted that if the effects of cations on zoospores involved a direct interaction of cation with anionic plasmalemma, the relative order of cation activity would correspond to one of these sequences.

The relative order of activity of the alkali-cations on encystment was K > Na > Cs > Li which is either sequence V or VI of Diamond and Wright. However, the order of effectiveness in causing cell damage did not correspond to any of the 11 possible sequences. Neither did the order of activity of the divalent cation series on encystment or viability correspond to any

of the sequences proposed by Diamond and Wright for these ions, and the effects of $Ca^{2+}$ differed qualitatively as well as quantitatively from the other divalent ions.

It is known that Phytophthora zoospores are repelled by many inorganic cations (Allen & Harvey, 1974; Cameron and Carlile, 1980). This negative chemotactic response occurs at different threshold concentrations for each cation. Comparison of our results with the studies on negative chemotaxy shows that for some cations, e.g. $H^+$, $Na^+$, $K^+$, $Li^+$, $Ca^{2+}$, the threshold concentration for inducing negative chemotaxy is close to the minimum concentration for inducing a detectable effect, either encystment or cell damage. For $Mg^{2+}$ and $La^{3+}$ there is no correspondence, $Mg^{2+}$ being less effective and $La^{3+}$ more effective in our system.

For the monovalent cation series, at least, the negative chemotactic response follows the Hoffmeister lyotropic series for cation exchange reactions. Therefore a general mechanism to explain the phenomenon of negative chemotaxy has been suggested whereby cations interact with the negatively charged zoospore membrane. This reduces the negative charge, thus altering the transmembrane potential and reducing flagellar activity (Cameron & Carlile, 1980). However, our data and also that of Cameron and Carlile for divalent cations indicates that superimposed on such a general ion-membrane interaction there are specific ion effects. This is to be expected, as it is known that, even where binding constants are similar, different cations modify membrane structure in different ways (Williams, 1972; Papahadjopoulos et al., 1979). Beyond this there is the further situation of site specific binding of which that of $Ca^{2+}$ has been most intensively studied (Whitfield et al., 1980).

The studies reported in this paper also have practical implications. Although it is hazardous to extrapolate from the laboratory to the field, the results do suggest that soil cation content could influence the behaviour

of P. cinnamomi zoospores directly. In soils with high $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$ or $Fe^{3+}$ levels, the cells would not remain in the motile stage for long and this, in turn, could influence the rate of dispersion. The levels of the above cations required to induce encystment are in the range of concentrations reported for soil solutions (Nye & Tinker, 1977), although the levels of $NH_4^+$ required for any effect on zoospore behaviour would not be expected in natural waters. Failure of $Al^{3+}$ to produce any effect on zoospore motility or viability argues against this ion playing a role in the disease distribution, although it has been shown that this ion does limit mycelial growth of the related P. capsici (Muchovej et al., 1980).

Although high cation levels reduce the viability of motile zoospores, once encystment has taken place in the absence of these cations the cells are more resistant to their effects. The results also show that zoospores of P. cinnamomi have a broad tolerance to pH. Only the extremes of the acid or alkaline range observed in soil might be expected to inhibit zoospore development. Similarly zoospores and cysts tolerate a broad range of temperatures, although surface soil temperatures might be expected to exceed 25° and fall below 10°, the limits of tolerance, on many occasions. Here too the cyst stage displays a greater tolerance of low temperatures than the motile stage, although it is a different spore form, the chlamydospore, which is the survival stage of this organism.

Acknowledgements

This work was supported in part by grants from the Victorian Forests Commission and from the Potter Foundation to B.R.G. P.N.B. was supported by a Commonwealth Postgraduate Research Award.

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TABLE 1. Divalent and Trivalent Cations: Effects on Viability and Encystment

| Addition | Concentration μM | % Encystment at 30 min | % Viability at 30 min |
|---|---|---|---|
| $La(NO_3)_3$ | 1 | 100* | 25±9 |
| $FeCl_3$ pH 5.5 | 15 | 92±8 | 79±8 |
| $FeCl_3$ pH 5.5 | 100 | 100 | 65±5 |
| $MnCl_2$ | 500 | 98±2 | 73±5 |
| - | - | 26±5 | 90±3 |

*$La^{3+}$ causes immobilization, but not encystment.

TABLE 2. Summary of Effects of Inorganic Cations on Zoospores

| Cation | Concentration mM | Response after 30 min % Immobilization | % Damage |
|---|---|---|---|
| - | - | 15 | 5 |
| $La^{3+}$ | 0.001 | 100 | 75 |
| $Fe^{3+}$ | 0.015 | 92 | 20 |
| $Al^{3+}$ | 0.030 | <20 | <10 |
| $H^{+}$ | 0.1 | 70 | 11 |
| $Mn^{2+}$ | 0.5 | 98 | 27 |
| $K^{+}$ | 5 | 95 | 30 |
| *$Ca^{2+}$, $Sr^{2+}$ | 20 | 90 | 5 |
| $NH_4^{+}$ | 30 | 85 | 50 |
| $Mg^{2+}$ | 30 | 50 | 60 |
| $Na^{+}$ | 30 | 35 | 20 |
| $Li^{+}$ | 30 | 20 | 65 |
| $Cs^{+}$ | 30 | 35 | 90 |

*$Ca^{2+}$-induced cysts germinate spontaneously.

TABLE 3. Effect of various salts on encystment and viability

| Addition | Concentration mM | % Encystment at 60 min | % Viability at 60 min |
|---|---|---|---|
| - | - | 16±5 | 95±4 |
| NaCl | 20 | 18±7 | 91±7 |
| | 40 | 79±7 | 64±12 |
| $NaNO_3$ | 20 | 39±11 | 91±4 |
| $Na_2SO_4$ | 20 | 74±11 | 70±8 |
| $NaH_2/Na_2HPO_4$ pH 6 | 20 | 51±15 | 88±7 |
| NaF | 20 | 33±7 | 67±8 |
| $Na(CH_3COO)$ pH 6.8 | 20 | 94±12 | 48±14 |
| KCl | 20 | n.d. | 56±10 |
| $NH_4Cl$ | 20 | n.d. | 45±16 |
| $CaCl_2$ | 20 | 100±12 | 96±2 |

TABLE 4. Effect of cations on cyst viability.

| Addition | Concentration mM | % Viability |
|---|---|---|
| Control | - | 84±6 |
| $Na^+$ | 50 | 80±7 |
| $K^+$ | 50 | 58±7* |
| $Li^+$ | 50 | 68±10 |
| $Cs^+$ | 50 | 3±3* |
| $NH_4^+$ | 50 | 62±4* |
| $Ca^{2+}$ | 30 | 100±3 |
| $Mg^{2+}$ | 30 | 97±3 |
| $Sr^{2+}$ | 30 | 80±4 |
| $Ba^{2+}$ | 30 | 90±2 |

Cysts were exposed for 40 min at 19°.

*Significant at level P = 0.05.

FIGURE LEGENDS

Figure 1. Effect of temperature on zoospores and cysts. Cell suspensions were held in 1 mM tris-succinate buffer, pH 6, for 2 h at the temperatures indicated. Results are expressed as the percentage of the total population which were viabile or motile. Error bars represent ± 1 S.D.

Figure 2. The effect of pH on zoospore viability. Zoospore suspensions in 1 mM tris-succinate buffer, pH 6, were held for 30 min, 19°C at the pH's shown and then assayed for motility and viability.

Figure 3. Effect of KCl and NaCl on zoospore encystment. Salts were added to zoospore suspensions, to give final concentrations as indicated, and cells were fixed with glutaraldehyde after 20 min, 19°C. No cell loss was detected in any samples.

Figure 4. Effect of $NH_4Cl$, CsCl and LiCl on zoospore encystment. Conditions as for Fig. 3 except incubation extended to 40 min.

Figure 5. Effect of salt concentration on zoospore viability. Conditions as for Fig. 3. Incubation period was 20 min $K^+$, $Na^+$ and $NH_4^+$ and 40 min for $Li^+$ and $Cs^+$. Viability was assessed following the addition of $Ca^{2+}$/V8 and incubating at 24°C, 3 h. No cell loss was detected in any sample.

Figure 6. Effect of divalent cations on zoospore immobilization. Conditions as for Fig. 4, except that $Mg^{2+}$-treated samples were incubated 90 min at 20°C prior to fixation. No cell loss occurred in $Ca^{2+}$, $Sr^{2+}$ or $Ba^{2+}$-treated samples, but was found in the presence of $Mg^{2+}$.

Figure 7. Effect of $La^{3+}$, $Fe^{3+}$, $Mn^{2+}$ and $Ca^{2+}$ on zoospore encystment. Conditions as for Fig. 3.

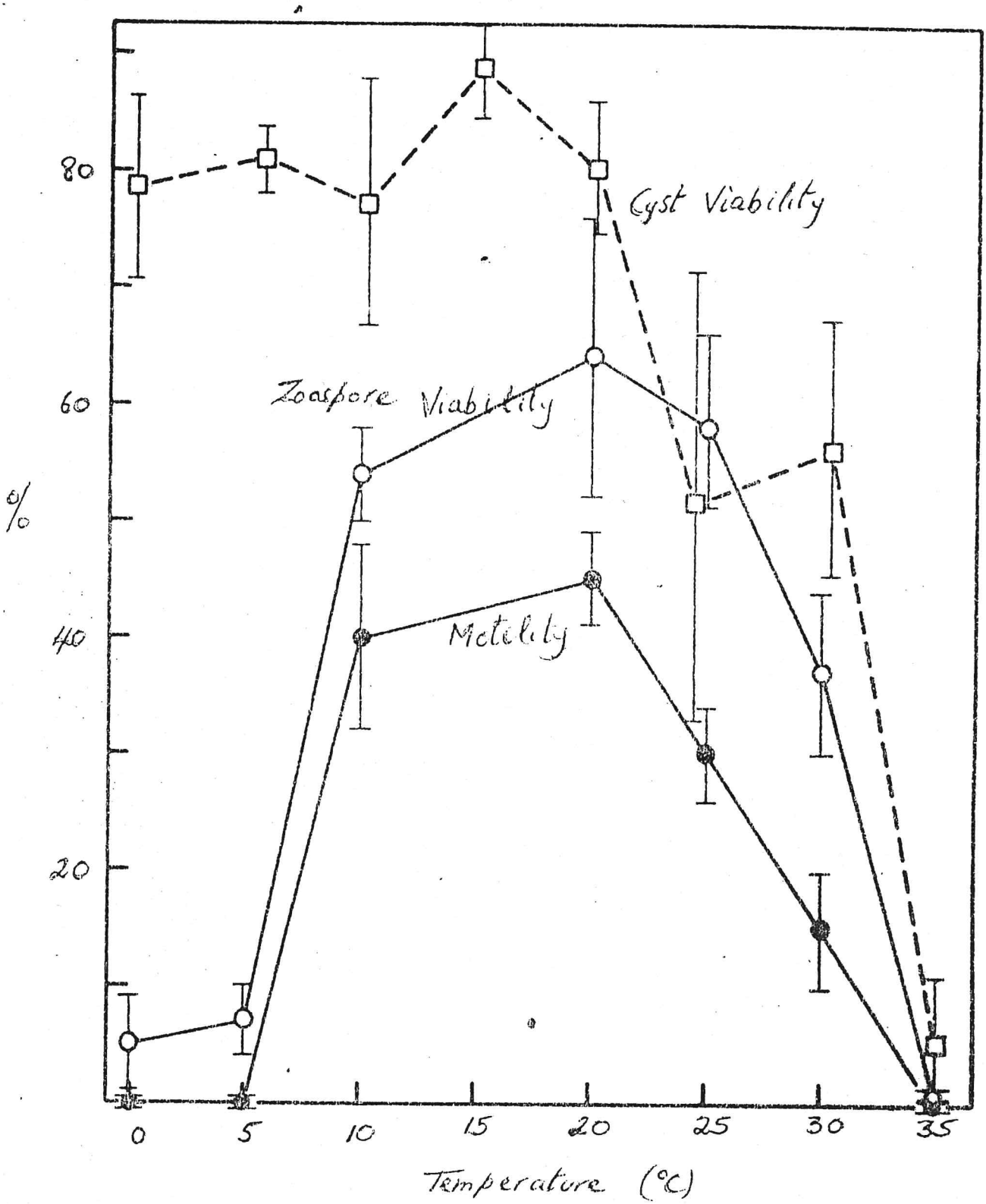

80
60
40
20
%
Cyst Viability
Zoospore Viability
Motility
0
5
10
15
20
25
30
35
Temperature (°C)

Fig-1

Figure 2

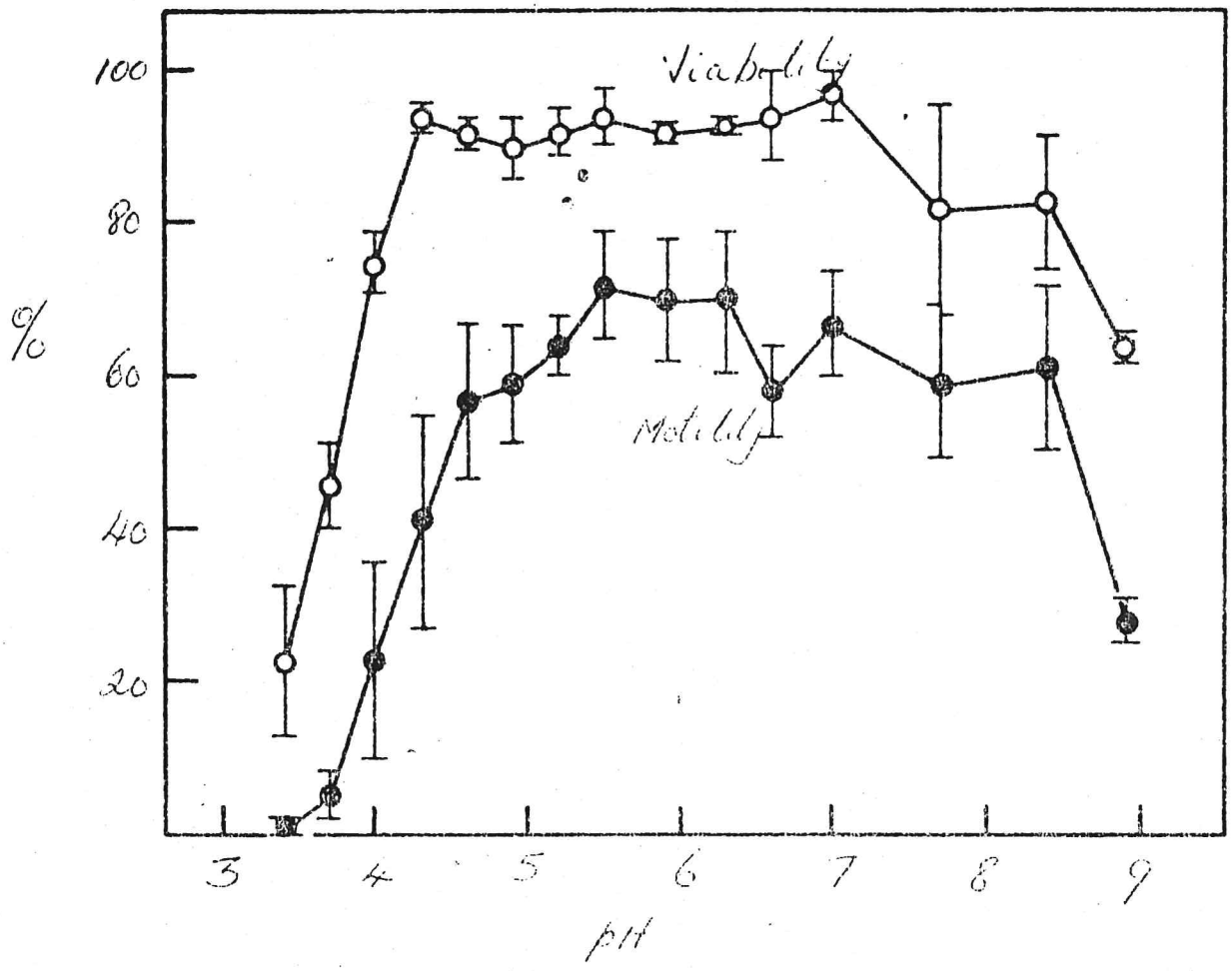


Figure 3

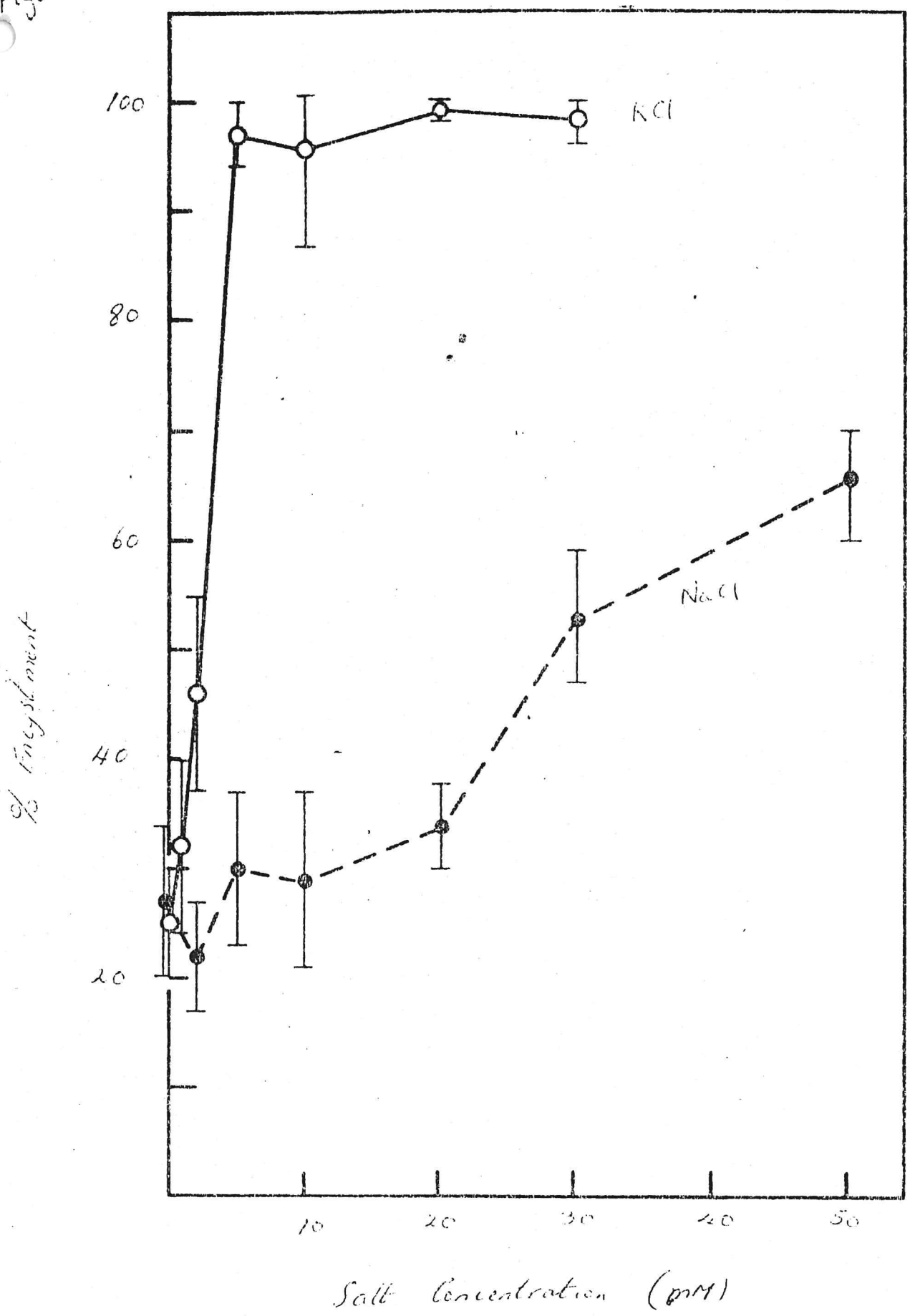

100
80
60
40
20
% Encystment
KCl
NaCl
10
20
30
40
50
Salt Concentration (mM)

Figure 4

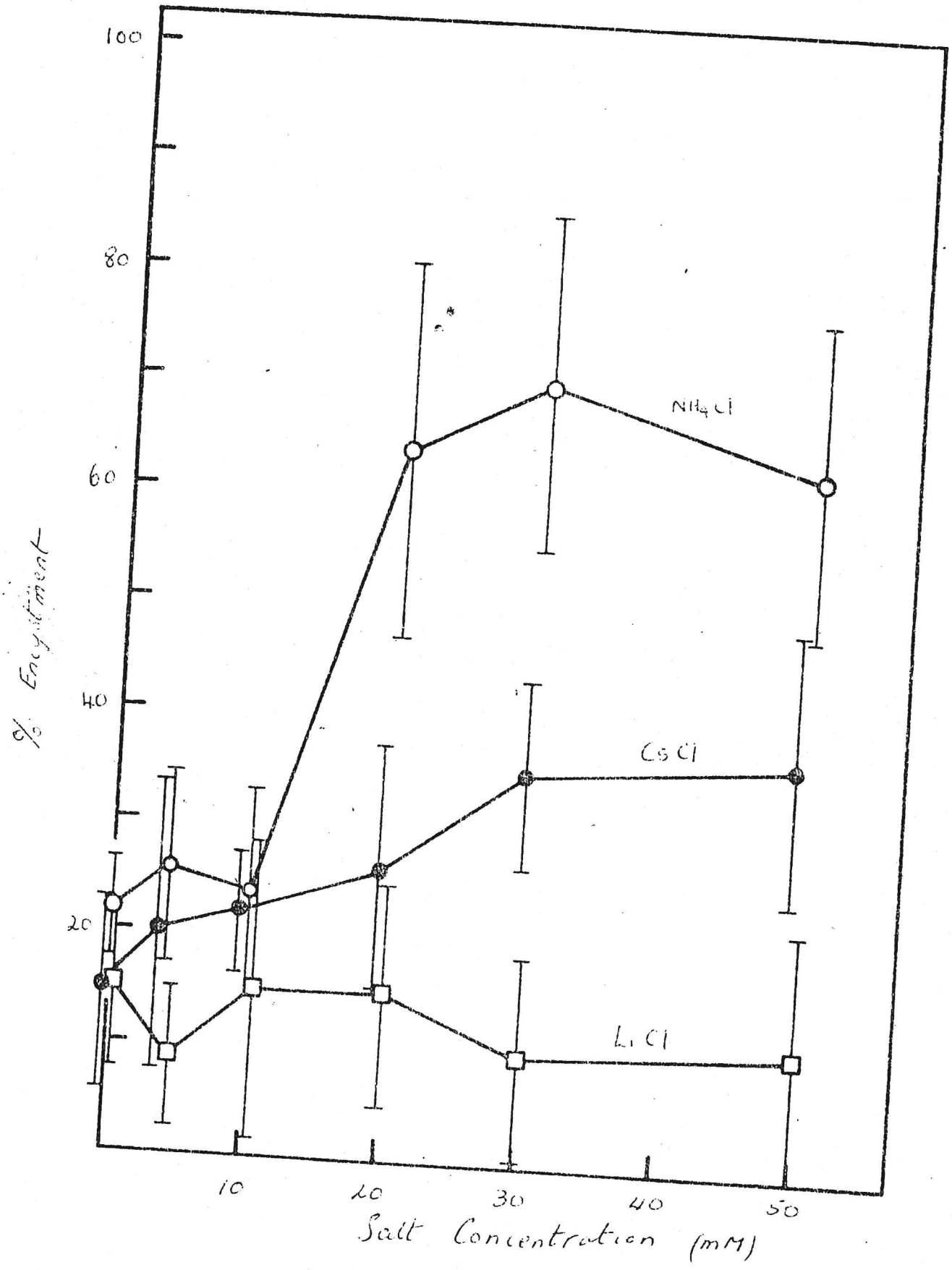

100
80
60
40
20
% Encystment
NH4 Cl
Cs Cl
Li Cl
10
20
30
40
50
Salt Concentration (mM)

Figure 3

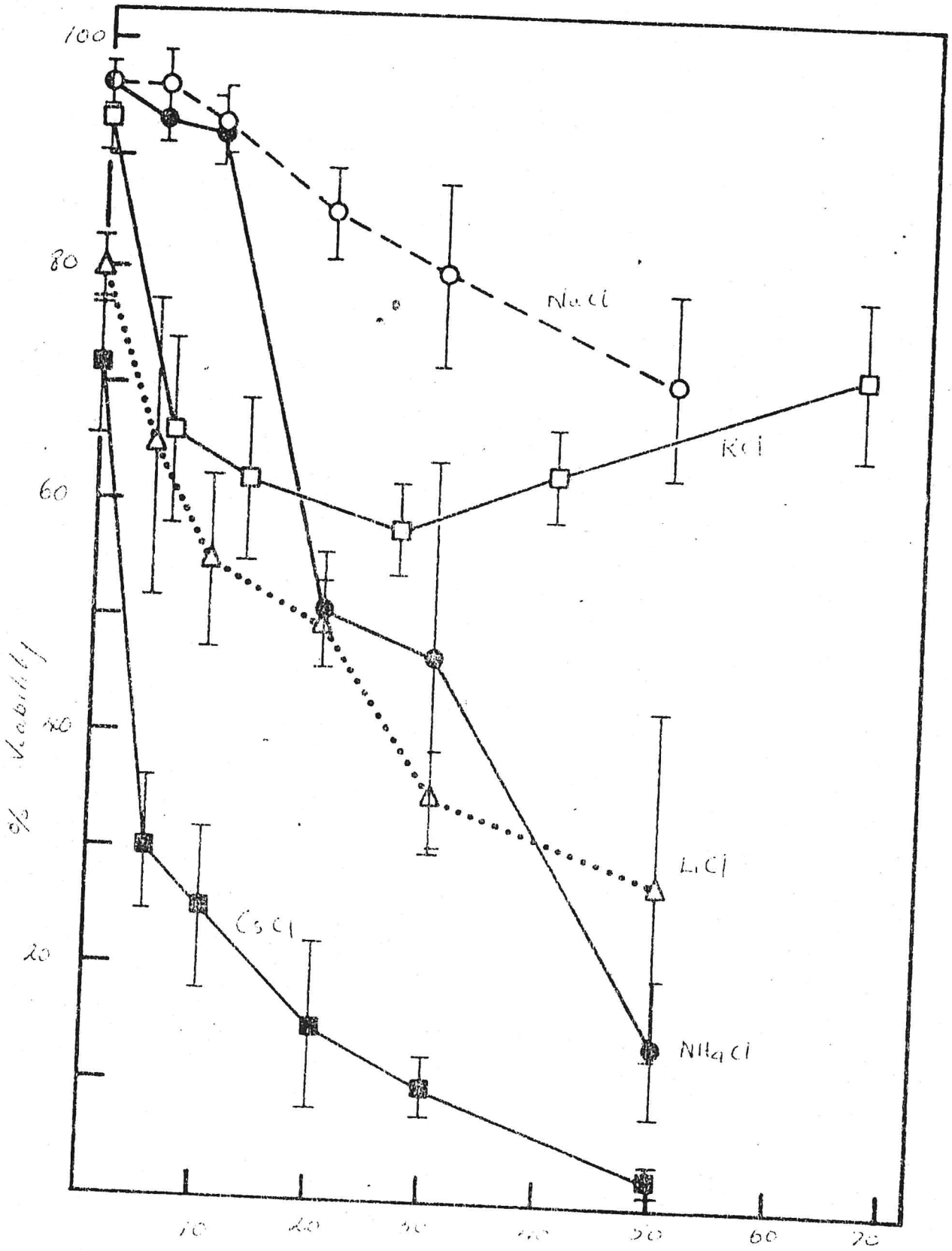
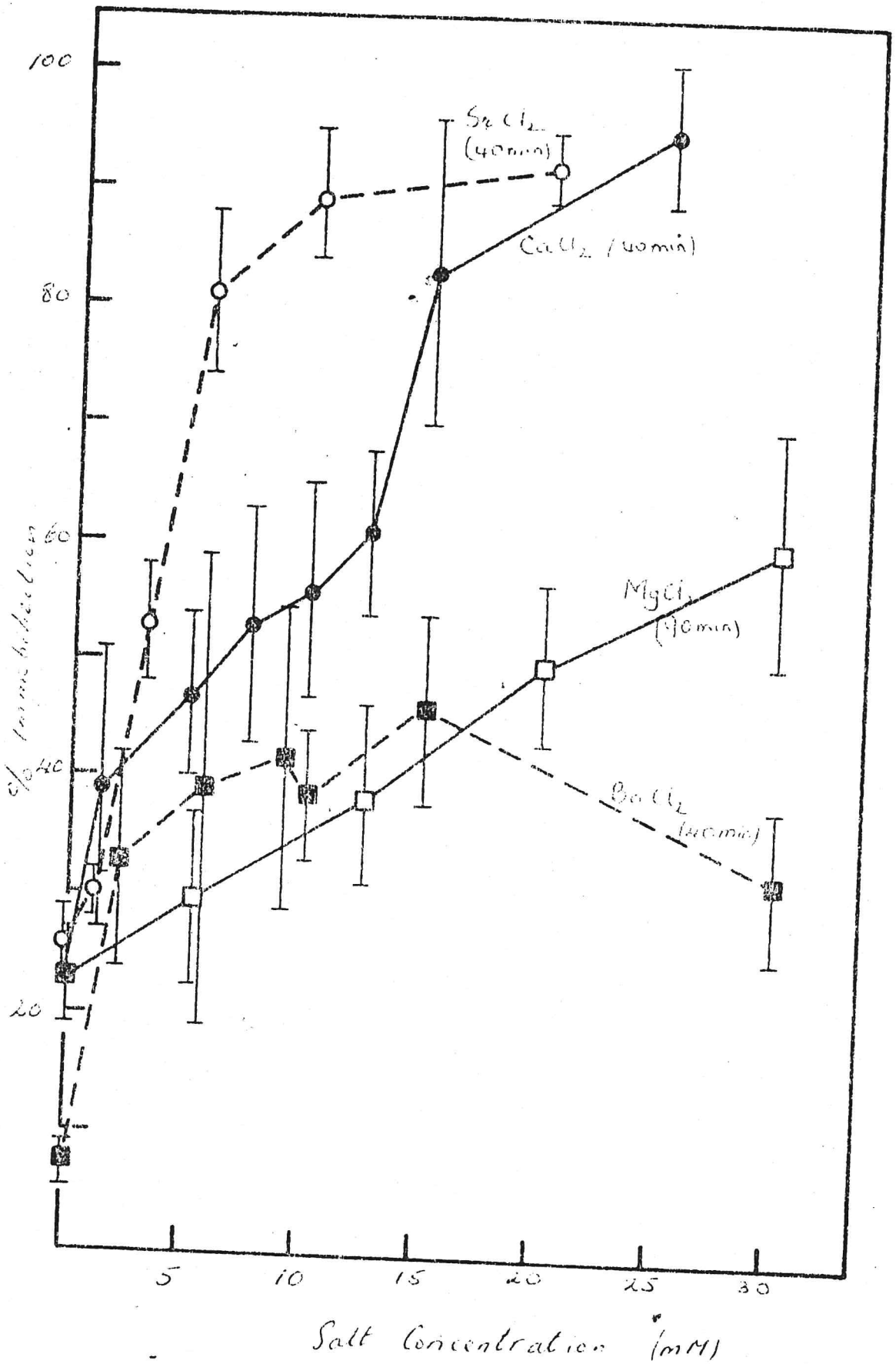
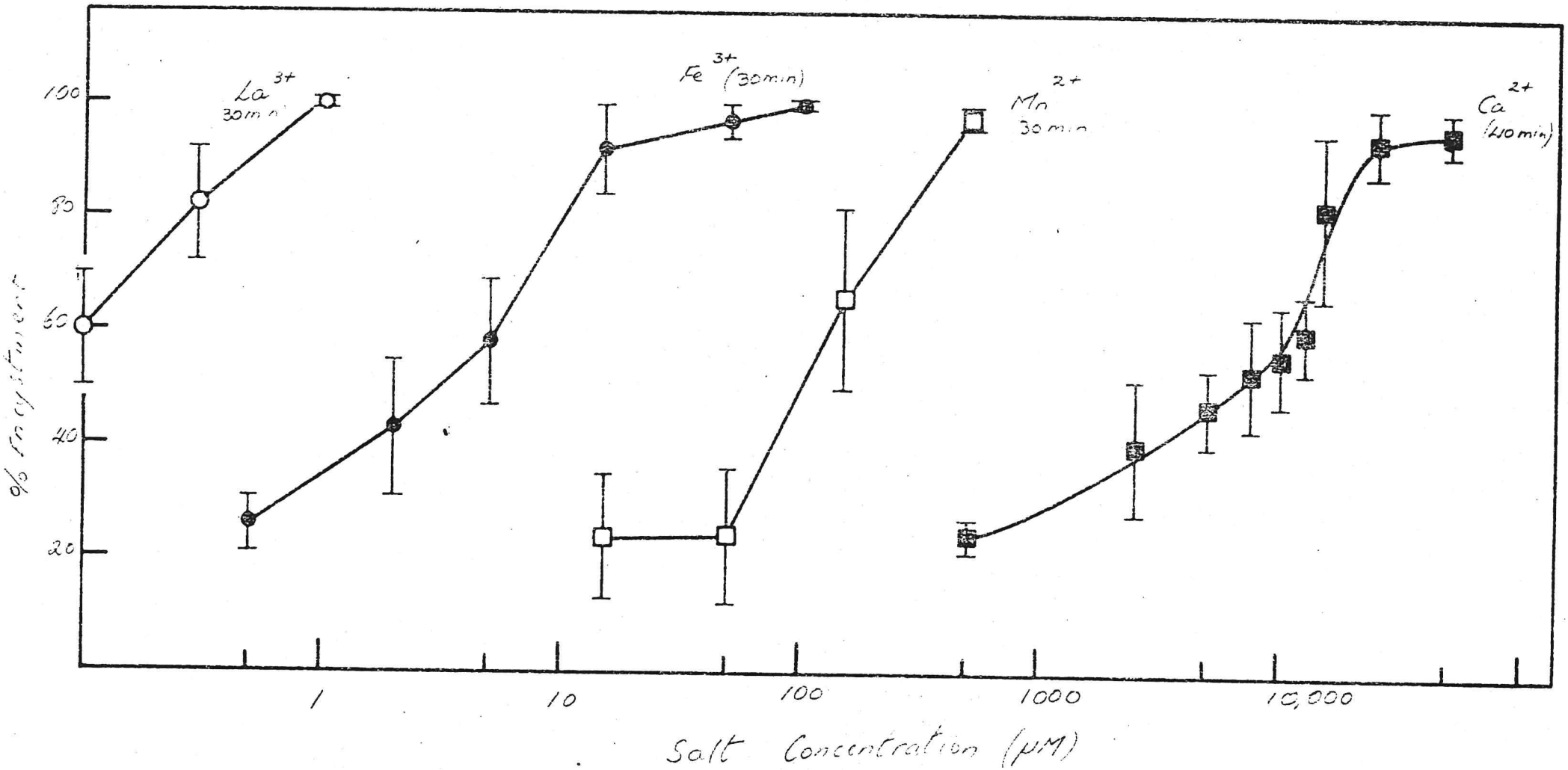

100
80
60
40
20
% Viability
NaCl
KCl
LiCl
CsCl
NH4Cl
10
20
30
40
50
60
70
Salt concentration (mM)

Figure 6



100
80
60
40
20
% Immobilisation
$SrCl_2$ (40min)
$CaCl_2$ (40min)
$MgCl_2$ (90min)
$BaCl_2$ (40min)
5
10
15
20
25
30
Salt Concentration (mM)

Figure 7.


100
80
60
40
20
% Encystment
La 3+ 30 min
Fe 3+ (30 min)
Mn 2+ 30 min
Ca 2+ (240 min)
1
10
100
1000
10,000
Salt Concentration (μM)