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## **DOCTOR OF PHILOSOPHY**

**Studies on the acceptability and digestibility of artificial diets by crustacea.**

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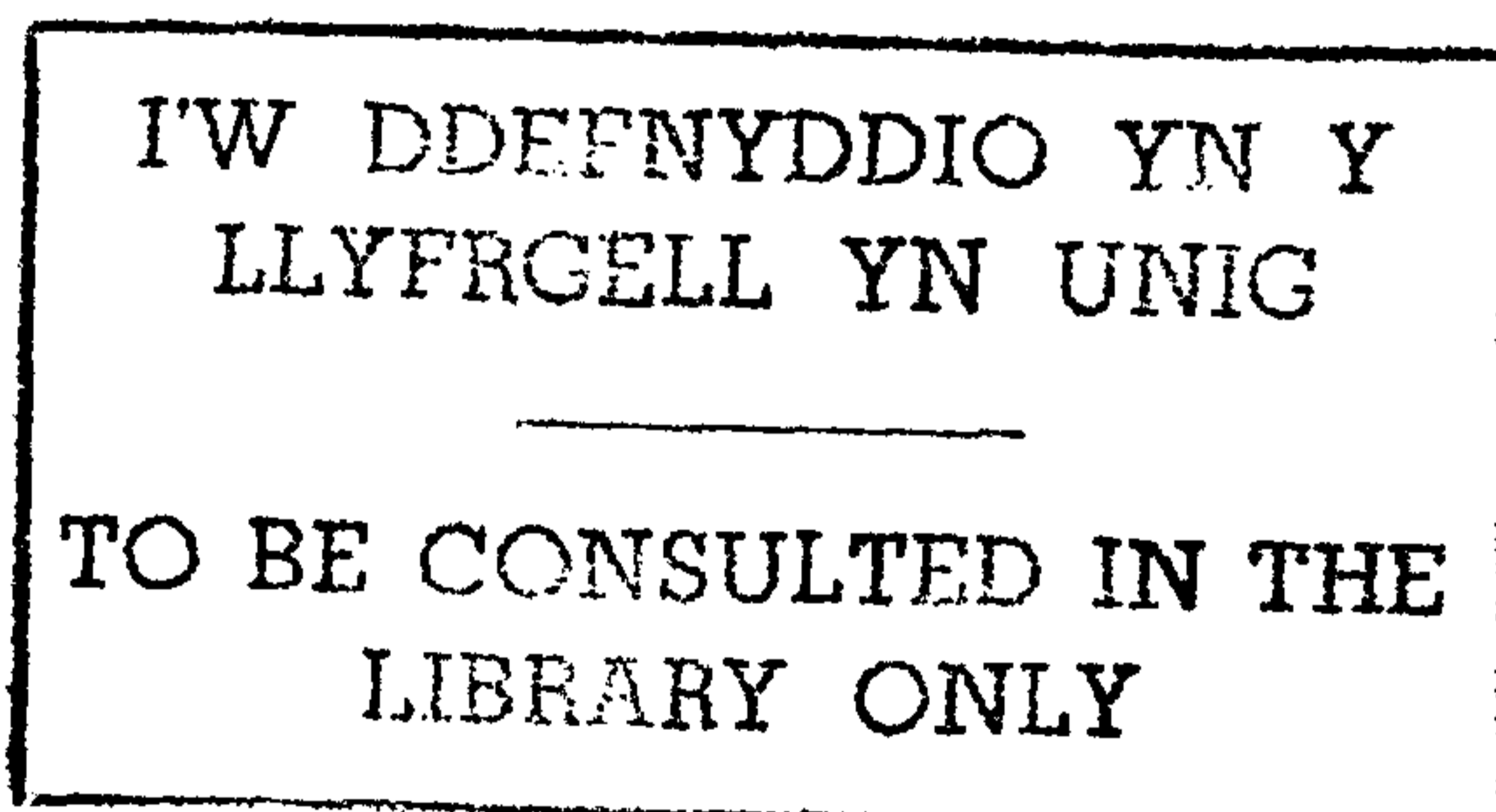
STUDIES ON THE ACCEPTABILITY AND DIGESTIBILITY OF ARTIFICIAL  
DIETS BY CRUSTACEA.

A Thesis submitted to the University of Wales

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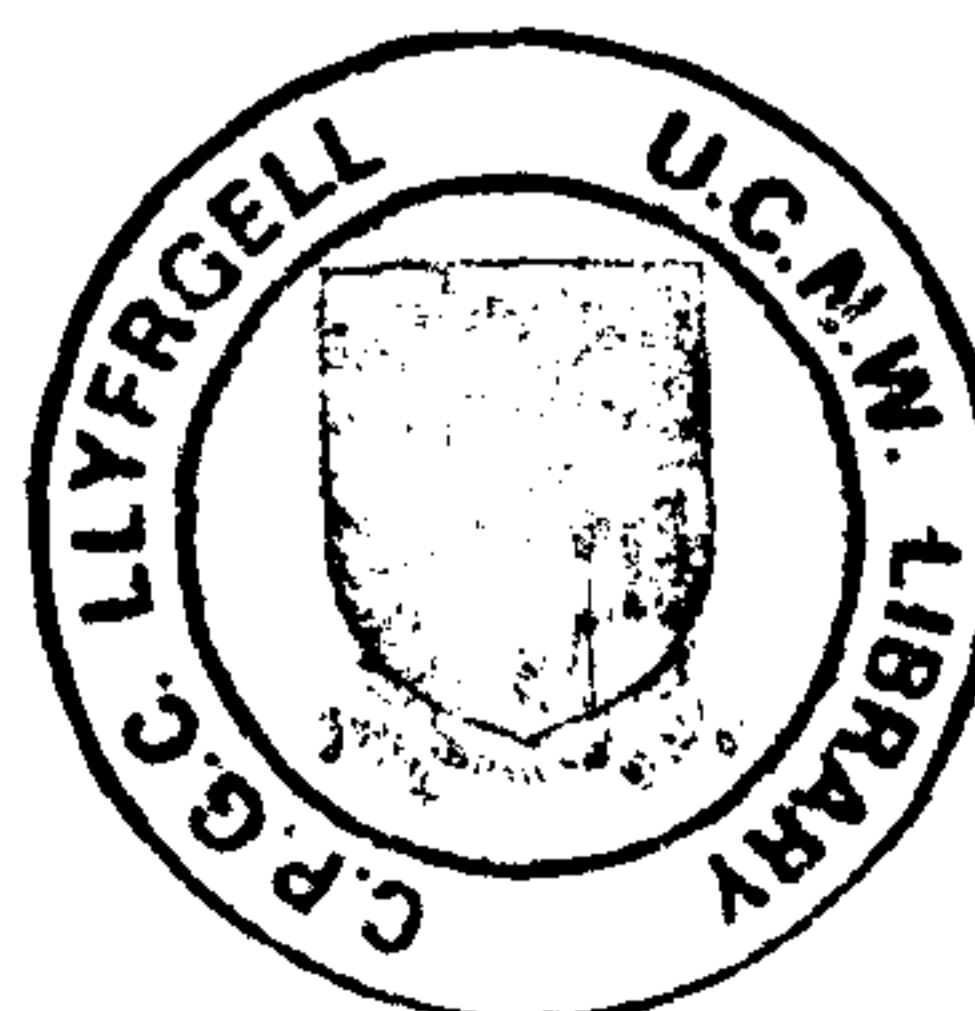
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Chapter 3 is available in *Aquaculture* 81.1 (1989): 27-45.

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Chapter 5 is available in *Aquaculture* 81.1 (1989): 13-25.

The details of Appendices 1 – 3 are listed in the Table of Contents of the thesis (pages 1 – 2).

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## SUMMARY.

### STUDIES ON THE ACCEPTABILITY AND DIGESTIBILITY OF ARTIFICIAL DIETS BY CRUSTACEA.

This work investigates the factors controlling production and determines the biophysical properties of cross-linked protein coated microencapsulated diets, with a view to enhancing their acceptability and digestibility to omnivorous and carnivorous larvae.

To estimate optimal feed levels required for rearing larvae of Penaeus monodon on microencapsulated diets, facilitate live transportation and add to the biological knowledge of the species, the relationship between metabolic rate/ temperature and energetics of the larval stages were investigated.

Also described, are laboratory growth and survival trials with P.monodon larvae fed on cross-linked protein microencapsulated diets. Success with P.monodon larvae, led to investigations on acceptability, growth and energetics of juvenile P.monodon reared on an artificial diet. For comparison, a similar line of approach was adopted with the carnivorous larvae of Homarus gammarus. Studies on the physiological energetics of the larvae were followed by investigations on acceptability, growth and digestibility of artificial diets fed to H.gammarus larvae. The final discussion attempts to correlate the contrasting larval growth/ survival results to the different energy strategies adopted by the larvae.

"What is written without effort is in general read without pleasure."

Samuel Johnson (1709 - 1784).

Johnsonian Miscellanies, Vol. II, p. 309.

"Give a man fish and you feed him for a day; teach a man to grow fish and you feed him for a lifetime".

Old Chinese proverb.

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Jones, D.A. and Kurmaly, K., 1987. Feeding and assimilation of Frippak and other feeds by crustacean larvae.

J. World Aquacult. Soc., 18:17.

11 APPENDIX 2.

\ Jones, D.A., Kurmaly, K. and Arshad, A., 1987. Penaeid shrimp hatchery trials using microencapsulated diets. Aquaculture, 64:133-146.

\ 12 APPENDIX 3.

Clark, J., Jones, D.A. and Kurmaly, K., (in press). Penaeid larval rearing on microencapsulated feeds. In: Volcker, C.M. and Volcker, A. (eds.), 1st Inter-American Congress of Aquaculture, 14-21 sept. 1986, Salvador, Brazil.

13 REFERENCES.

## Introduction.

The Food and Agricultural Organisation of the United Nations (FAO) predict that by early next century world demand for fish and shellfish, 130 million tonnes, will outstrip the world fishery catch of 90 million tonnes, which is currently approaching the maximum sustainable yield of the oceans (FAO, 1982). The developing gap between fishery supply and market demand necessitates that any increase in supply to world markets will have to come from an alternative source. On this basis Nash (1987) predicts that aquaculture production will reach 22.2 million tonnes by the year 2000 and account for 25% of total world fisheries.

Strong international demand for prawns has already fully exploited wild shrimp resources (1.72-1.82 million tonnes) such that supply is unable to satisfy demand (Robinson, 1982). Increasing demand and subsequent high prices paid for prawns (US \$7-15kg<sup>-1</sup>), has resulted in a dramatic expansion in commercial cultivation of marine and freshwater prawns (Bailey, 1988). For example, in Ecuador, cultured prawn production rose from 4,800 tonnes in 1978 to 23,360 tonnes in 1983, an increase of 500% (McPadden, 1985). The phenomenal success of Ecuador has fuelled private sector investment which presently plays the major role in establishing prawn mariculture systems worldwide. In several Asian countries investment is encouraged by tax credits, subsidised loans and infrastructural development (Coche, 1982). Overall, worldwide production from farms has risen steadily from <1000 tonnes in the late sixties to >300,000 tonnes in 1985 and although increasing, supply is continually unable to meet demand (Nash, 1988).

The rate at which supply can be intensified is presently controlled by the availability of postlarval prawns, disease and development of cost effective feeds. The absence of reliable sources of wild postlarvae has given rise to the development of a separate hatchery industry, specialising in the culture of the planktonic larval stages of penaeids. Although rearing techniques have improved since the pioneering work of Hudinaga (1969), a feeding regime calling for the provision of algae during the protozoal stages and zooplankton for the mysis and early postlarval stages has been almost universally followed (Heinen, 1976). Current algal and zooplankton feeds used in penaeid larval culture are presented in Table 1.

While a large number of algal species may be used as larval food there are presently several limiting problems confronting aquaculturists. In applications where the quantity of required algae is relatively modest ( $<10\text{g m}^{-2} \text{d}^{-1}$ ) laboratory techniques are well developed, higher algal requirements however have been difficult to realise, owing to expense (up to 40% of running costs: Laing and Helm, 1981) and reliability (Liao, 1984). Although, research aimed at improving technology and reducing costs of algal production is continuing (Benemann et al., 1980; De Pauw et al., 1983; Pruder, 1981), consistent production of large quantities of algae ( $60\text{--}200\text{g m}^{-2} \text{d}^{-1}$ ) remains an elusive goal. Production is often erratic, difficult to maintain and prone to collapse (Persoone and Claus, 1980).

In addition, variability in the nutritional quality of algae particularly in essential polyunsaturated fatty acids (PUFA), has been shown to seriously impede commercial larval productivity (Scott and Middleton, 1979; Kanazawa et al., 1977; Langdon and Waldock, 1981; Watanabe et al., 1983; Lei and Su, 1985). This problem is further exacerbated by the generally accepted concept that the nutritional quality of algae is significantly influenced by chemical and physical parameters of the culture environment (Mykkestad and Haug, 1972). Nutritional variations in algae have been correlated with changes in nutrient concentrations, salinity, temperature, light and growth rate (Fisher and Schwarzenbach, 1978; Goldman et al., 1979; Rhee, 1980; Scott, 1980; Webb and Chu, 1983; Teshima et al., 1983b; Fabregas et al., 1985, 1986).

Although there is an extensive list of zooplankton species that could serve as food for penaeid larval stages (Watanabe et al., 1983), the practical choice of a reproducible, readily available, live food is limited to rotifers e.g. Brachionus plicatilis and the branchiopod Artemia. The former is frequently used in commercial penaeid hatcheries (Liao et al., 1983; Fujita, 1980; Girin, 1979; Yap, 1979), widespread use however, is limited to small operations due to difficulties in mass rotifer culture (Schluter et al., 1987; Snell et al., 1987) and the requirement for quality feeds, high in PUFAs, during rotifer culture (Watanabe et al., 1983).

Unlike other live food organism, for mysis and later penaeid stages, which have to be maintained in continuous culture, Artemia cysts constitute the most practical food

(Sorgeloos, 1980). However, problems and constraints related to its use have been reported by several workers and include, the presence of cyst contaminants effecting tank hygiene (Gilmour et al., 1975; Austin and Allen, 1982), rapid post-hatch depletion of Artemia nauplii lipids (Wickins, 1976; Benijts et al., 1976), and nutritional variability between Artemia strains (Watanabe et al., 1980, 1983; Leger et al., 1985, 1986). In consequence pre-feeding Artemia nauplii on diets high in w3 PUFA, prior to use, is often necessary (Watanabe et al., 1983; Jones et al., 1984) but disadvantageous, since enriched Artemia are often too large to be used for penaeid mysis stages. Alternatives to Artemia as a food source, primarily due to concern over availability and price (\$ 70 kg<sup>-1</sup>) have been investigated by Kahan and Apel (1975) and use of the nematode Panagrellus sp. was recommended. Wilkenfeld et al., (1984) however, recently concluded that Panagrellus sp. was of low nutritional value to prawn larvae and difficult to mass produce.

While prawn larvae at present are routinely reared on live foods there is a consistent inability to duplicate results throughout the larval season (Cook and Murphy, 1969; Mock et al., 1980a,b). Survival has been shown to range from 5-44% for P.japonicus (Hudinaga and Miyamura, 1962; Hudinaga and Kittaka, 1967; Shigueno, 1975), 1-100% for P.monodon (Liao and Huang, 1972; Platon, 1978; Beard and Wickins, 1980) and 0-77% for P.stylirostris (Mock et al., 1980b; Wilkenfeld et al., 1983, 1984) when grown on live feeds. Despite these problems, hatcheries are dependent on live feeds due to the lack of suitable alternatives. Subsequently, the need to develop an artificial alternative, that is readily acceptable

(Moller et al.,1979; Ache, 1982), neutrally bouyant (Meyers, 1971), water stable (Jones et al.,1979a), digestible (Gibson, 1983) and meets the larval nutritional requirements (Jones et al.,1979b) has intensified in recent years, not least, to improve the economic efficiency of the owner operator with limited resources.

In view of the acceptability of artificial particles by zooplanktonic organisms (Jones et al.,1974; Poulet and Marsot, 1978; Fernandez, 1979; Donaghay and Small, 1979) various workers have developed either unprotected (processed natural products and microgels) or protected (microencapsulated particles) artificial diets (Table 2). Processed natural products including frozen, dried or freeze dried algae, yeast and shellfish crumbles (Brown, 1972; Sick and Beaty, 1975; Murai and Andrews, 1978; Mock et al.,1980b) have had limited success in hatcheries, primarily due to cell lysis, leaching and subsequent bacterial fouling of the water column. Although microgels are simply prepared by mixing dietary components with a selected gelling agent e.g calcium alginate, carageenan, zein or agar (Teshima et al.,1983a; Kanazawa et al.,1982; Langdon, 1983; Levine et al.,1983; Langdon et al.,1985) and at relatively low cost, they exhibit poor water stability (Adron et al.,1974; Jones et al.,1979a) and often, due to the high particle concentrations required for filter feeders, accelerate culture collapse (Gatesoupe et al.,1977; Goldblatt et al.,1980). Several commercial microgel products are available (e.g Argent, Nippai and Kyowa-hakko), none to date however, has successfully replaced live feeds used in commercial penaeid hatcheries.

The use of the process of microencapsulation to feed artificially compounded diets to filter feeding invertebrate larvae was first suggested by Meyers et al.,(1972). Packaging solids or liquids into small ( $0.5\mu\text{m}$ - $500\mu\text{m}$ ) protective capsules provides a micro-delivery system designed to release its contents in a predictable manner (Chang et al.,1966). The variety of techniques available for microencapsulating larval diets, may be categorised into two principal classes, phase separation (Chang et al., 1966; Langdon 1980) and interfacial polymerisation reactions (Chang et al., 1966; Jones et al.,1974; Kondo et al.,1976). Since aqueous components are rapidly lost during preparation, the use of phase separation techniques in developing artificial larval feeds has been limited to the production of microcapsules enclosing non-aqueous nutrients (Langdon, 1980). The latter technique of interfacial polymerisation however, modified by Jones et al.,(1974, 1979a,b) to produce nylon-protein coated microcapsules, has successfully been used to provide a nutrient-delivery system and determine the nutrient requirements of planktonic larval organisms (Jones et al.,1979a; Kanazawa et al.,1982; Sakamoto et al.,1982; Chu et al.,1987).

Unfortunately, while nylon-protein microcapsules are well established as a research tool, in addition to their tendency to form aggregates (Jones and Gabbott, 1976; Levine et al., 1983) there are several problems limiting their commercial use; they are permeable to low molecular weight compounds which may accelerate bacterial fouling of the larval culture (Jones and Gabbott, 1976), they are liable to lose lipid in the organic phase during encapsulation (Jones et al., 1979a;

Langdon, 1980), they may contain toxic surfactants e.g Tween 20, which have been shown to interfere with larval feeding behaviour (Moller et al., 1979) and finally, due to the fragility of the nylon-protein walls they are unable to withstand drying.

In consequence, Jones et al., (1984) revised the method of encapsulation to produce dried, cross-linked protein coated, non-toxic microcapsules, using a stronger acid chloride and eliminating nylon and Tween 20 (British patent Nos: 79437454 and 2103568). The aim of this project was to investigate factors controlling production and to determine the biophysical properties of cross-linked protein coated microencapsulated diets, with a view to enhancing their acceptability and digestibility to omnivorous and carnivorous planktonic larvae, Penaeus monodon (Fabricius) and Homarus gammarus (Linnaeus).

To estimate optimal feed levels required for rearing P.monodon larvae on microencapsulated diets, facilitate live transportation and add to the biological knowledge of the species, the relationship between metabolic rate/temperature and energetics of larval stages were investigated. Also described, are laboratory growth and survival trials with P.monodon larvae fed on cross-linked protein microencapsulated diets. Success with P.monodon larvae, led to investigations on acceptability, growth and energetics of juvenile P.monodon reared on an artificial diet. For comparison, a similar line of approach was adopted with the carnivorous larvae of H.gammarus. Studies on the physiological energetics of the larvae were followed by investigations on acceptability,

growth and digestibility of artificial diets fed to H.gammarus larvae. The final discussion attempts to correlate the contrasting larval growth/ survival results to the different energy strategies adopted by the larvae.

Table.1. Summary of current algal and zooplankton feeds used to rear penaeid larvae.

Species	Feed type	Density ml <sup>-1</sup>	Stage	Survival (%)	Author
<u>Penaeus</u>	<u>Skeletonema</u>	6-10x10 <sup>5</sup>	Z1-M1	50.0	Cook (1967)
<u>aztecus</u>	<u>costatum.</u>				
"	<u>Artemia.</u>	10	Z3-PL1		"
<u>P.aztecus</u>	<u>S.costatum.</u>	2-4x10 <sup>5</sup>	N6-M1	75.0	Mock and
	<u>Artemia.</u>		M1-PL5		Murphy (1970)
<u>P.indicus</u>	<u>Thalassiosira</u>	4x10 <sup>3</sup>	N6-Z1	95.6	Emmerson
	<u>weisflogii.</u>	7x10 <sup>3</sup>	Z1-Z3		(1980,1984)
	"	2x10 <sup>3</sup>	Z3-M3		
<u>P.japonicus</u>	<u>Chaetoceros</u>	5-30x10 <sup>5</sup>	Z1-Z3	35.8	Hirata et al.,
	<u>rigidis.</u>				(1975)
<u>P.japonicus</u>	Diatoms.	5-8x10 <sup>3</sup>	Z1-PL20	25.0	Hudinaga and
	<u>Artemia.</u>				Kitaka (1975)
<u>P.japonicus</u>	<u>Chaetoceros</u>	5-7x10 <sup>4</sup>	Z1-PL1	65.0	Jones et al.,
	<u>gracilis.</u>				(1979a)
	<u>Artemia.</u>				

<u>P.japonicus</u>	<u>Chaetoceros</u>	5-7x10 <sup>4</sup>	Z1-PL1	95.0	Kanazawa et al., (1982)
	<u>gracilis.</u>				
	<u>Artemia.</u>	15			
<u>P.marginatus</u>	<u>Chlorella sp.</u>	2.5-3.0x10 <sup>5</sup>	Z1-PL8	60.0	Gopalakrishnan
	<u>Artemia.</u>	1-3	Z3-PL10		(1976)
<u>P.merquiensis</u>	<u>Tetraselmis</u>	75x10 <sup>3</sup>	Z1-M1	58.0	Beard et al.,
	<u>suecica.</u>				(1977)
	<u>Artemia.</u>	2-5	M1-PL1		
<u>P.monodon</u>	<u>S.costatum.</u>	5x10 <sup>3</sup>	Z1-M3	35.0	Liao and Chin
	<u>Artemia.</u>		M1-PL5		(1980)
	<u>Rotifer.</u>		M1-PL1		
<u>P.monodon</u>	<u>Cylindrotheca sp.</u>	8-15x10 <sup>5</sup>	Z1-Z3	60.0	Aquacop
	<u>Tetraselmis sp.</u>	1-4x10 <sup>4</sup>	Z3-PL2		(1977)
	<u>Rotifer.</u>	10	M1-PL2		
<u>P.monodon</u>	<u>Tetraselmis</u>	1-5x10 <sup>4</sup>	Z1-Z3	79.0	Beard and
	<u>suecica.</u>				Wickins (1980)
	<u>Isochrysis</u>	2-25x10 <sup>4</sup>	Z1-Z3		
	<u>galbana.</u>				
	<u>Artemia.</u>	5	M1-M3		

<u>P.vannamei</u>	<u>C.gracilis.</u>	3-10x10 <sup>4</sup>	Z1-M1	80.0	Simon (1978)
<u>P.stylirostris</u>					
	<u>C.gracilis.</u>	3-10x10 <sup>4</sup>	Z1-M1	85.0	Simon (1978)

Table.2. Use of artificial diets to rear invertebrate filter feeding larvae.

a.Unprotected artificial diets

Species	Feed type	Result	Author
<u>Macrobrachium</u>	Freeze dried	11% survival	Sick and
<u>rosenberghii</u>	catfish.	to metamorphosis	Beaty (1975)
<u>M.rosenberghii</u>	Freeze dried	<2% to	Murai and
	oyster and trout	metamorphosis	Andrews (1978)
	feed.		
<u>Palaemonetes</u>	Freeze dried	26.7% survival to	Sandifer and
<u>pugio</u>	squid.	metamorphosis	Williams (1980)
<u>Penaeus</u>	Freeze dried and	45% to mysis I	Brown (1972)
<u>aztecus</u>	frozen diatoms.		
<u>P.japonicus</u>	Soyabean cake	Poor growth and	Hirata et al.,
	crumble.	survival	(1975)
<u>P.japonicus</u>	Microgel:diet B	67% to PL1	Jones et al.,
	(Kanazawa et al.,		(1979)
	1977).		

<u>P.japonicus</u>	Microgel:diet B.	94% to PL1	Kanazawa et al., (1982)
<u>P.setiferus</u>	Frozen <u>Skeletonema</u> <u>costatum</u> and <u>Artemia</u> .	80% to PL1	Mock et al., (1980a)
<u>Eurypanopeus</u> <u>depressus</u>	Microgel:calcium alginate and rotifers.	50% to megalopa	Levine et al., (1983)
<u>Crassostrea</u> <u>virginica</u>	Microgel particles. exhibited 75% growth of algal fed controls		Langdon and Siegfried (1984)
<u>C.virginica</u>	Microgel particles. 52% growth of algal fed controls		Langdon and Bolton (1984)
b.Protected artificial diets			
<u>Brachionus</u> <u>plicatilis</u>	Nylon-protein microcapsules.	prolonged culture >5 days resulted in decrease of population	Teshima et al., (1981)

<u>Artemia</u>	"	35% survival to adult	Sakamoto et al., (1982)
<u>M. rosenberghii</u>	"	survival to stage IV only	Jones et al., (1975)
<u>P. merguensis</u>	"	survival to mysis I	Jones et al., (1975)
<u>P. japonicus</u>	"	50% survival to PL1	Jones et al., (1979)
<u>P. japonicus</u>	"	78% survival to PL1	Kanazawa et al., (1982)
<u>Eurypanopeus depressus</u>	"	83% survival to megalopa	Levine et al., (1983)
<u>Crassostrea gigas</u>	Nylon-protein microcapsules.	Promoted spat growth	Gabbott et al., (1975)
<u>C. gigas</u>	Gelatin/acacia microcapsules.	Enhanced spat growth	Langdon and Waldock (1981)
<u>C. virginica</u>	Gelatin/acacia and lipid walled microcapsules.	25% survived after 25 days	Chu et al., (1987)

Preparation, factors effecting construction and biophysical properties of cross-linked protein microcapsules.

## INTRODUCTION.

Filter feeding zooplanktonic organisms require dietary particles that are readily accepted, ingested and digested. Hence, in addition to studies on factors influencing yield, particle size, wall texture and thickness are also important criteria to be considered in the design of microencapsulated diets. Particularly as particle size and wall texture control ingestion whilst wall thickness and composition determines digestibility.

To optimise utilisation efficiency of dietary particles by filter feeding organisms, investigations were designed to determine the biophysical properties; pH, wall permeability and bouyancy, of microencapsulated diets. The digestive fluid of most marine crustaceans operate optimally between pH range 5-7 (Vonk, 1960; Van Weel, 1970; Barker and Gibson, 1979). Since by-products such as succinic and hydrochloric acid, from the interfacial polymerisation reaction, may well reduce encapsulated dietary pH (McGinity et al., 1981; Cuff et al., 1986), steps were taken to determine the effect, if any, of processing on the pH of the dietary components and larval gut pH.

Nowhere is nutrient leaching more of a hazard than in artificial diets for marine filter feeding larvae (Meyers, 1979). The size of the mouthparts requires use of small food particles with a very high surface area/ volume ratio and mode of feeding necessitates the continuous presence of a high concentration of particles. Leaching of nutrient components from the dietary vehicle in such circumstances undoubtedly contributes rapidly to the deterioration of water quality, by

stimulating bacterial growth (Jones et al.,1979a). In consequence, the rate of release of water soluble protein compounds was determined for several diets.

The tendency for artificial diets to settle out of suspension continues to present a major obstacle to efficient utilisation of microencapsulated diets by filter feeding organisms (Clark et al.,in press). Settlement not only reduces availability of dietary particles to filter feeding larvae, but also results in the formation of clumps of particles which are too large to be ingested once resuspended. Hence, a prerequisite to efficient utilisation of artificial diets by filter feeding larvae is the use of suitable forms of agitation designed to maintain particles in suspension without damaging larvae. While several mechanisms have been used (Jones et al.,1979a,b; Levine et al.,1983), as yet, no thorough investigation has been undertaken to determine their efficiency and ascertain their drawbacks. The present work evaluates the efficiency of a commercially available system of MF Ventura aerators and then compares several systems constructed in the laboratory, with a view to designing a more efficient commercial system.

## MATERIALS AND METHODS.

The process of microencapsulation can be sub-divided into three successive stages (Figure 1):

- a) emulsification of the amine
- b) interfacial polymerisation (cross-linking)
- c) isolation of microcapsules.

In the course of the initial stage of emulsifying the amine, three modifications have been implemented to the original process, described by Chang et al., (1966), which resulted in non-biodegradable membranes. Nylon has been substituted by the polyamine haemoglobin, its free amine groups enable it to cross-link in the presence of a diacid to form a capsule wall. A single organic solvent, cyclohexane, as opposed to a binary mixture, is used. Cyclohexane has a density of between 0.7 and 1.1, is not miscible with water, has a lower affinity for lipids and does not denature proteins. The reaction takes place at room temperature (20°C), increasing the effectiveness of cross-linking.

During the second stage, microcapsule walls are formed by interfacial cross-linking, specifically by means of a bifunctional acylating agent in which two functional groups (chloride ions) are separated by an aliphatic juxtannuclear chain, as in succinyl dichloride. The cross-linking agent dissolved in cyclohexane is added to the reaction mixture. The reaction relies on the insolubility of the diacid in water and the appreciable partition coefficient of the amine towards the organic phase. As the rate of diffusion of the amine out of the aqueous phase does not exceed the rate of reaction, polymerisation takes place entirely at the interface. If the

reaction is too brief, a leaky, fragile, macroporous membrane is formed. On the other hand, prolonged contact between amine and diacid will yield a thick membrane or an undigestible capsule, with an excess of low molecular weight polymers on the organic side (Chang, 1972).

Microcapsules are isolated from the reaction mixture by quenching the reaction with excess solvent and submitting the suspension of microcapsules to a series of washes. Chang et al., (1966) used Tween 20 to facilitate transfer from the organic to the aqueous phase. Since Tween 20 is toxic (Moller et al., 1979), a successive series of rinses in cyclohexane with 2% lecithin (w/v) followed by a wash in 15% gelatin solution (Jones et al., 1984) is essential. Dried microcapsules were obtained by lyophilisation.

To study factors influencing microcapsule yield, size, wall texture and thickness, several modifications were made to the procedure outlined above and in Figure 1. In addition to the solvent system used, the concentration of Span 85, speed of agitation, acid chloride type and concentration, were each altered to investigate their effect on microcapsule construction. For each reaction, the microcapsule mean diameter of 50 freeze-dried capsules and yield, estimated gravimetrically on tared glass fibre filter papers (G.F.C. Whatman Ltd) after drying at 60°C for 24 h, were determined. Wall texture, thickness and shape were studied from electron micrographs taken with a Hitachi 520 S.E.M, using freeze-dried gold coated microcapsules mounted on aluminium stubs. Trials were run in duplicate for each factor investigated.

Biophysical properties of microencapsulated and microparticulate diets.

The effect of processing on the pH of dietary components was tested using the following available commercial microencapsulated diets; AR103, AR118, AR120 and CD227 (Frippak Feeds U.K. Ltd). Laboratory prepared microencapsulated diets were produced using a 20% w/v solution of a 1:1 ratio of haemoglobin and Kanazawa diet B (Kanazawa et al., 1971), blended and sieved to less than 20  $\mu\text{m}$ . Microparticulate diets of less than 20  $\mu\text{m}$  were prepared following Kanazawa et al., (1971, 1982). For each trial, 100 mg of diet (<20  $\mu\text{m}$ ) were homogenously distributed in 200ml of U.V irradiated, 0.2  $\mu\text{m}$  cartridge filtered seawater, and aerated for 72 h. pH was read after 30 minutes and subsequently every 24 h using a Phillips PW 9410 digital pH meter. Parallel experiments were conducted in distilled water to avoid the buffering effect of seawater.

The quantity of proteinaceous nutrients leached from gently aerated microencapsulated (CD136, CD301 and CD434) and microparticulate diets bound in carageenan and zein (Kanazawa et al., 1971, 1982), in conditions and concentrations similar to those described above, was determined colorimetrically using Bio-Rad (BIO-RAD laboratories). At regular time intervals filtered (G.F.C filter paper) dietary solutions were analysed for dissolved protein after complexing with Bio-Rad, using a  $1\text{cm}^3$  cuvette, read with a SP 500 spectrophotometer set at OD 595 nm. Soluble protein was estimated from a standard curve determined for bovine serum albumin (Sigma Chemical Co). Bacterial attachment to the microcapsules and growth in the

water column was determined using the acridine orange fluorescent technique on millipore, 0.2  $\mu\text{m}$  filtered, samples (Parsons et al., 1984).

To determine the efficiency of the available commercial particle suspension system of three MF aerators (Venturator Ltd), a circular pool (PY 1236: Polar pools Ltd) of volume 9621 l (3.66m diameter by 0.91m deep) and three piston pumps (6 p.s.i and 15 c.f.m. each: B.V.C. Ltd) for aeration, were set up in a semi-enclosed area (Plate 1a,b). The MF aerators lift and circulate water using the principle of raising water by air injection, based on the weight equilibrium of fluids. Air injection, through MF aerator port holes, produces an air-water mixture of less dense fluid that rises, overflows and consequently transports water. MF aerators were modified to increase their effectiveness with the attachment of skirts and short eduction cylinders (Figure 2). Before deployment, in addition to a conditioning period of 24 h in seawater, the iron balast of all three MF aerators was coated with fibre-glass resin to prevent iron oxidation and subsequent spoilage of pool water. To determine the optimal working height, methylene blue dye (10% w/v) was introduced at four sites A, B, C, and D at depth and below the surface (Figure 3) while a single MF aerator unit was in operation at one of several heights tested, 4, 10, 25 and 50cm off the pool floor.

Settlement trials were conducted with MF aerator units positioned at optimal height and at the apices of an equilateral triangle (116 cm) centred in the pool in raw seawater. For each trial the microencapsulated diet was rehydrated for 0.5h in filtered (0.2 $\mu\text{m}$ ) seawater and then

distributed homogenously throughout the pool. 200ml aliquote samples were siphoned at regular time intervals from five sites A-E (Figure 3), at a depth of 40cm. Microcapsule concentrations were determined from two 10 ml sub-samples removed from each aliquote. Using a haemocytometer the mean rate of particle settlement was estimated. Initial trials in non-turbulent water were followed by trials using MF aerators injected with air either continuously or discontinuously.

Suspension systems investigated in the laboratory with a view to developing an efficient system for commercial use, include the following:

Non-mechanical: aeration systems

Aerator            with airstones, small bore tubes (1.0 mm I.D) and large bore tubes (5.5 mm I.D) in 2l round bottom flasks and immhoff cones.

Airlift pump (Figure 4)

Mechanical systems:

Paddle stirrer    ( $3.85 \text{ cm}^2$  paddle blade; 40 r.p.m)

Plunger            (Figure 5)

For each system, pre-weighed, rehydrated (15 minutes at 100 r.p.m) artificial diets, sieved to less than  $30 \mu\text{m}$  were introduced to give a particle concentration of  $50 \mu\text{l}^{-1}$ . At regular time intervals aliquote samples of 10 ml were siphoned and the concentration, and particle size distribution

determined using a coulter counter Model ZB (Coulter Electronics). Trials were replicated and investigations were initially conducted in unmixed water and later followed by observations on the various agitation devices.

## RESULTS.

### Factors effecting microcapsule construction.

#### Effect of span 85 and mechanical agitation on microcapsule construction.

For a fixed speed setting of 200 r.p.m. increasing concentrations of span 85 result in an increase in yield and decrease in mean particle size (Table 1). For a fixed concentration of span 85, the mean particle size produced can be controlled by the speed of agitation (Table 2). At agitation speeds of 1500 r.p.m obtained using an homogeniser, with blunt blades, and 3 ml of span 85, microcapsules of less than 20  $\mu$ m, which are ideal for planktonic filter feeding larvae (Plate 2: see electronmicrograph A), can be produced.

#### Effect of acid chloride concentration on microcapsule construction.

For each solvent system used, with the exception of chloroform, an increase in the acid chloride concentration of sebacoyl chloride results in a corresponding increase in wall thickness, suggesting that more of the dietary component is used in wall formation (see Table 3 and plates 2:B to 4:D). Decrease in size and an alteration in microcapsule shape, from spherical to angular, are also evident with cyclohexane and a 1:4 (v/v) mixture of chloform:cyclohexane (Table 3). This is possibly related to the heat of reaction which often reached 50-60°C, with increasing acid chloride. The increased heat of reaction gives rise to a rate that exceeds and disrupts the uniform diffusion rate of the amine, producing dense non-sphericle particles (Plate 3:F).

Effect of different solvent systems, for a given acid chloride concentration, on microcapsule construction.

Changing the solvent system when using 0.5 ml sebacoyl chloride, from cyclohexane to a binary mixture of 1:4 (v/v) chloroform:cyclohexane, to 1:1 (v/v) chloroform:cyclohexane and on to chloroform, results in a decrease in mean particle size, from 320  $\mu\text{m}$  to less than 60  $\mu\text{m}$  and an increase in wall thickness, from 3-10  $\mu\text{m}$  to over 30  $\mu\text{m}$  (See Table 3 and plates 2:B to 5:D). Whilst, use of cyclohexane results in microcapsules having wrinkled thin and brittle walls (Plate 2:B and C) distinct from the internal contents of the capsule, chloroform, which has a higher solubility in water, results in granular thick-walled microcapsules with evidence of cross-linking permeating right through the internal diet (Plate 4:F; Plate 5:A and B).

Effect of various acid chlorides and solvent systems on microcapsule construction.

Succinyl dichloride dissolved in cyclohexane reacts with the amine at the interface to produce small (85  $\mu\text{m}$ ) smooth walled microcapsules, cross-linking is intense at the interface and results in the deposition of a wall of 10-30  $\mu\text{m}$  in thickness (see Table 4 and plate 5: E and F). With chloroform, larger (150  $\mu\text{m}$ ), smooth thin walled (4-8  $\mu\text{m}$ ), microcapsules are produced (Plate 6:A and B). The apparent reversal in microcapsule characteristics with the two solvents between succinyl dichloride (M.W. 154.99) and sebacoyl chloride (M.W. 239.14), is due to the increased reactivity and rate of hydrolysis of the former.

Interfacial polymerisation with malonyl chloride (M.W.140.95), close to the molecular weight of succinyl dichloride, gives similar results, small ( $<80\ \mu\text{m}$ ) and large ( $200\ \mu\text{m}$ ) particles are obtained with cyclohexane and chloroform, respectively (see Table 4 and plates 6:C to F; Plate 7:A). Oxalyl chloride (Table 5), however, yields large ( $250\ \mu\text{m}$ ), thin, brittle walled microcapsules, which are easily ruptured, with both solvents (see Table 4 and plate 7:B to F).

Biophysical properties of microencapsulated diets.

Effect on pH of culture media.

Results presented in Table 6a show that all microencapsulated diets tested severely depress the pH of seawater. This is attributable to the acidic by-products of the interfacial reaction, X-ray diffraction analysis on microencapsulated diets showed a large  $\text{Cl}^-$  peak. A smaller drop in pH is shown when microparticulate diets are immersed in seawater. Interestingly, while the pH of seawater containing microencapsulated diets is relatively stable after 24 h at around pH 7.8, the pH of seawater containing microparticulate diets fluctuates between 7.4 and 8.0 (Figure 6a).

In comparison to seawater, the pH of distilled water is not only more severely depressed with the addition of microencapsulated diets, but does not recover (Table 6b, Figure 6b). Pre-washing microencapsulated diets for 15 minutes however, limits the drop in pH. The addition of a microparticulate diet initially raises the pH of distilled water to 6.6, over the duration of the experiment this value

drops to 5.7 .

#### Wall permeability

Percentage exudation rates of water soluble protein (Table 7a) show conclusively the benefit of delivering diets in encapsulated form. Average rate of release after 24 h, from microencapsulated diets, is one third lower than the rates given for microparticulate diets, which are similar in dietary formulation. Although rates of release from microencapsulated diets do increase, most probably due to bacterial attack (Table 7b), CD301 and CD434 encapsulated diets manage to retain over 50% of their water soluble protein. Table 7b suggests that microparticulate diets are particularly vulnerable to bacterial attack, it is likely that ineffective sterilisation techniques during processing and packaging contribute to the explosive bacterial growth.

Suspension trials using a commercially available agitation system.

Comparative regression analysis on the natural log percentage rate of settlement of microencapsulated particles (AR 114 <80  $\mu\text{m}$ , CD136 60-90  $\mu\text{m}$ , CD136 90-150  $\mu\text{m}$ ) in non-turbulent water, indicates no significant difference, at the 5% level, between particle size and rate of settlement (Figure 7a). Results on the rate of settlement of microencapsulated particles in turbulent water, with the MF aerators operating continuously and discontinuously, converted to natural log are presented in Figure 7b. Comparative regression analysis on the data indicates that the discontinuous method of operating the piston pumps increases

the efficiency of the MF aerators in preventing particle settlement, particularly with particles of  $<90\ \mu\text{m}$ . Both systems however, show a 50% loss in particle concentration after only 3 h.

Suspension trials using laboratory designed agitation systems.

Without agitation

Particle size distributions of artificial diets shown in Figure 8, indicate that microparticulate diets contain a greater fraction of large sized particles. Figure 9 shows a plot of the natural log of percentage number of artificial dietary particles remaining in suspension against time (minutes), in still water. Comparative linear regression analysis indicated a significant difference, at the 5% level, between the rate of settlement of microencapsulated and microparticulate diets, the former group had a lower fall velocity.

Non-mechanical agitation: Aeration system

Several aeration systems were investigated and the natural log of the percentage rates of settlement of microencapsulated diets against time are plotted in Figure 10. Trials with Imhoff cones were unsuccessful and are not included, observations revealed that microcapsules soon formed a scum around the rim of the cones. Comparative linear regression analysis on the results indicates that with the exception of system C (Figure 10), which allowed particles to settle out of suspension rapidly, there was no significant difference, at the 5% level, between the systems investigated.

## Comparison between mechanical and non-mechanical agitation systems.

The rate of settlement of a microencapsulated diet (CAR 003) in mechanical and non-mechanical agitation systems is shown in Figure 11. No linear comparisons were made due to the obvious difference in settlement patterns. Clearly, agitation systems dependent on aeration are less efficient than mechanical systems, indeed the paddle systems maintain approximately 90% of the diet in suspension for periods up to 10 h while the plunger device maintains 50% in suspension for up to 16 h. Figure 12 however, shows a shift in the particle size distribution after 10 h when using mechanical systems, suggesting that although particles stay in suspension for longer periods, the mean particle size increases as a result of clumping.

## DISCUSSION.

### Factors effecting microcapsule construction.

Although interfacial polymerisation appears superficially to be simple and is from a preparative standpoint, the chemistry and theory have yet to be fully understood (Bradbury and Crawford, 1972). Hence, only a few tentative reasons for the results given are discussed. The corresponding rise in yield of microcapsules with increase in span 85 concentration has been tentatively attributed to the increased solubility of the amine in the organic phase, due to the decrease in interfacial tension, facilitated by the emulsifier (Chang et al., 1966; Benita et al., 1984). As a rise in agitation speed or shear energy alone, does not produce microcapsules able to withstand transfer into the aqueous phase, span 85 not only helps to emulsify the two phases, but also has an important role in stabilising the newly formed emulsion and nascent membranes. By lowering the surface tension it enables cross-linking to proceed more effectively (Chang 1972; Levy et al., 1982). However, Koishi et al., (1969) obtained microcapsules without the presence of a surfactant by using polyphthalomide.

The mass of cross-linked porous material obtained with a 1:1 (v/v) chloroform: cyclohexane binary mixture (Plate 4:A,B,C and D) is possibly due to uneven diffusion and incomplete cross-linking of the amine, a result of the low concentration of acid chloride (Bradbury and Crawford, 1972; Benita et al., 1984) and solvent ratio (Chang et al., 1966). Strong microcapsules obtained with 1:4 (v/v) chloroform:cyclohexane suggest that this mixture is the

optimum binary system for the two solvents using sebacoyl chloride. It is possible that the rate of diffusion of the amine is closely balanced by the rate of reaction at the interface in a 1:4 (v/v) mixture (Chang et al., 1966). Choice of solvent system is consequently of vital importance, particularly as it determines the partition coefficient of the amine, its solubility, diffusion and reaction rate.

Unlike most acid chlorides which have a low solubility in aqueous solutions, oxalyl chloride which is a short chain low molecular weight aliphatic diacid, has a high rate of hydrolysis (Roberts and Caserio, 1965; Saul, 1972). Quite likely the weak walled microcapsules produced with oxalyl chloride can be attributable to its high rate of hydrolysis. The explosive rate of hydrolysis may interfere with interfacial cross-linking to such an extent that little of the amine reacts with the acid chloride.

Investigations show that the construction of microcapsules, their yield, diameter, wall texture and strength are greatly dependent on the surfactant concentration, speed of agitation, concentration and type of acid chloride, and the solvent system used. Generally, the interactions or side reactions between the various components, other than the interfacial polymerisation reaction, can significantly effect construction of microcapsules by their influence on surface tension, solubility of the amine and reactivity of the acids. Similar findings regarding the importance of side reactions have been reported by Chang et al., (1966) and Koishi et al., (1969), and through their use enhance the possibility of producing a microencapsulated diet

tailored to the requirements of filter feeding organisms.

#### Biophysical properties of microencapsulated diets.

In order to investigate the effect that microencapsulated diets may have on the pH within larval guts, a high concentration of artificial diet was used in seawater to simulate the possible effect. Not suprisingly, the buffering capacity of seawater enables it to better withstand perturbations in pH caused by the addition of artificial diets, than distilled water (Skinner, 1965). The initial drop and fluctuation in pH however, which is followed by a rise to normal (pH 8.0) after 24 h, suggests that seawater is unable to compensate rapidly. Previous work with nylon-protein microcapsules has shown that the pH of encapsulated diets can reduce enzymatic activity by 40% (McGinity et al., 1981; Wood and Whately, 1982). Whilst the low pH caused by the addition of microencapsulated diets into the water may well inhibit certain digestive enzymes within the guts of filter feeders, this effect on pH is nullified with a 15 minute pre-wash.

Although, it has been acknowledged that nutrient dissolution is a major problem with artificial diets (Meyers 1973,1979), few workers have considered its consequences before drawing conclusions from feeding trials using artificial diets (Kanazawa et al.,1971,1972,1982; Teshima et al.,1986). Investigations with conventional uncoated compounded feeds have shown that water soluble vitamins, free amino acids and certain minerals (Murai and Andrews 1975; Hilton et al.,1977; Goldblatt et al.,1979; Slinger et al.,1979) are rapidly lost through leaching. Losses of between 5-20%, 17-27% and 55-67% for pantothenic, folic and

ascorbic acids respectively, occurred through leaching from crumbled trout pellets after only 10 seconds in agitated water (Slinger et al., 1979).

Caution has also been expressed on the use of nylon-protein and ethyl cellulose microcapsules (Gabbott et al., 1975; Goldblatt et al 1979). Indeed, Chang et al., (1966) and Gabbott et al., (1975) suggested that they are only ideally suited for the delivery of macromolecules and are unsuitable for water soluble vitamins or amino acids. Permeability characteristics of microencapsulated diets have been described in terms of the diffusion process of solute through the membrane (Lee and Kondo, 1984; Uno et al., 1984) with the release rate dependent on molecular weight (Zhou and Chang, 1988). Data presented on leaching rates from protein coated microencapsulated diets suggests that unlike earlier capsules (Jones et al., 1974) or microbound diets, the present range of microcapsules, particularly CD301 and CD434 which have their water soluble nutrients emulsified in lipid prior to encapsulation (Baueja et al., 1986), efficiently retain the bulk of their water soluble protein component, for up to 24 h.

Additionally, present results point to a controlled release of nutrients from micro-encapsulated diets, in contrast to microparticulate diets. Zhou and Chang (1988) have also reported similar observations with microencapsulated diets incorporating prostaglandin  $E_2$ . This can be of great benefit since it not only delays the onset of explosive microbial growth, but may simultaneously stimulate ingestion if attractants are incorporated into the diet (Carr 1978; Zimmer-Faust and Michel, 1980). Hence, microencapsulated

diets, unlike microparticulate feeds, can provide a unique vehicle for the controlled release of feeding and gustatory attractants to filter feeding organisms. Subsequent rapid and positive food intake assists in reducing the residual time spent exposed to dissolution and enables more accurate determinations of the nutrient requirements of filter feeding larvae.

Observations on the commercially available suspension system (Figure 2) suggest that water carrying capacity of an MF aerator is positively correlated to its depth at operation. The range of times recorded for the dye to reach the MF aerator indicates however, that suction produced by the MF aerator is uneven; improper ballast packaging around the air ports may be responsible. Suspension trials with a variety of particle types and sizes resulted in a series of deposited rings of particles around each MF aerator, a consequence of interference between bottom incoming and surface outflowing water, which neither the skirt nor extended eduction pipe were able to overcome. After 3-5 h microcapsules were observed to adhere to each other, the pool wall and to debris in the water column. Adsorption of polyvalent ions, polypeptides, polysaccharides and bacteria, present in the unfiltered seawater, on to microcapsules most likely contributed to the increasing stickiness of the capsules (Floodgate, 1972; Paerl, 1975).

In certain instances a considerable number of grape-like clusters or fluidised masses of microcapsules were observed, within only 10 h. Clumping on this scale hindered resuspension of particles, resulted in particles too large to

be ingested and propagated the tendency to clump to freshly introduced microcapsules. Although the degree of clumping of microencapsulated diets will undoubtedly vary depending on the water filtration systems used and time of year, MF aerators appear unable to maintain more than 50% of particles in suspension for longer than 3 h. While the discontinuous use of piston pumps increased the efficiency of the MF aerators, the units failed to maintain the bulk of particles in suspension owing to their inability to cover the entire pool floor. An increase in operating units, even if economical, would be of little use since clumping would still be a problem. The proclivity to aggregate is not a characteristic unique to microcapsules, in a parallel study, particles such as carmine, silica and graphite were also shown to clump within hours of immersion in raw seawater.

Laboratory suspension trials show that with the exception of AR103, which is part of the first batch of diets produced by Frippak Feeds, microencapsulated feeds will remain in suspension for longer periods than microparticulate diets. This is not unexpected, since the microparticulate diets consist of a greater number of irregularly shaped particles. Angular particles have relatively lower drag coefficients and therefore are likely to fall more rapidly (Douglas, 1973).

Laboratory trials also suggest that mechanical devices are more efficient than non-mechanical devices in maintaining particles in suspension, if only for the initial 10 h. This is primarily due to the latter's inability to cover the entire vessel area, the mechanical devices used, operated close to the vessel floor, reducing the possible occurrence of dead

spaces. After 10 h however, the efficiency of mechanical devices to maintain particles in suspension appears to wane and particle clumping, reflected in the shift to large sized particles results in a drop in the suspended particle concentration (Figure 12). Once large clumps are formed no practical device is available for maintaining particles in suspension.

In conclusion, all the devices tested have drawbacks, however the cheapest to install and operate, in view of the variety in hatchery tank design (Liao et al., 1983), are aeration devices. Aeration systems can rapidly be modified to suit all types of tanks (Jones et al., 1987) indeed, directed water aerators have been used successfully in Taiwanese flat bottom rectangular tanks. The inability of aeration devices to cover the entire vessel area throughout the larval cycle, preventing settlement of the diet, can be overcome with the adoption of a feeding regime calling for more than one feed per day.

Table 1. Effect of span 85 on size ( $\mu\text{m}$ ) and yield (g) of microcapsules, at 200 r.p.m.

Volume of span 85 in 25 ml of cyclohexane (ml)	Mean diameter size of 50 microcapsules ( $\mu\text{m}$ )	Mean dry weight of yield (g)
0	-	-
1	280	0.23
2	160	0.21
3	105	0.56
4	118	0.41

Table 2. Effect of mechanical agitation (r.p.m.) on size ( $\mu\text{m}$ ) and yield (g) of microcapsules in 3 ml of span 85.

Speed of magnetic follower (r.p.m)	Mean diameter size of 50 microcapsules ( $\mu\text{m}$ )	Mean dry weight of yield (g)
200	340	0.53
400	105	0.51
800	67	0.46
1500*	17.5	0.49

\*= homogeniser

Table 3. Effect of sebacoyl chloride concentrations and different solvent systems on microcapsule construction.

Volume of sebacoyl chloride in 10 ml of solvent. (ml)	Solvent system	Wall thickness ( $\mu\text{m}$ )	Particle shape	Particle diameter ( $\mu\text{m}$ )	Wall texture and description of microcapsule.
0.5	cyclohexane	3-10	sphericle	>320	Wrinkled thin outer wall, distinct from internal contents (Plate 2:B and C).
3.0	"	10-16	sphericle	<100	Smooth walled microcapsules (Plate 2:D and E).
0.1	1:4 (v/v) chloroform: cyclohexane	4-6	sphericle	>500	Smooth strong walls with few flake attachments (Plate 2:F; Plate 3:A and B).
0.5	"	4-8	sphericle	>300	Wall heavily folded and fragile (Plate 3:C,D and E).

3.0	"	10-15	sphericle/ angular	<40	Few sphericle particles present, wall peppered with small particles (Plate 3:F).
0.5	1:1 (v/v) chloroform: cyclohexane	4-8	all shapes	>150	Mass of cross- linked porous material of no particular shape Plate 4:A,B,C and D).
3.0	"	5-15	sphericle	>150	Smooth walls encapsulating layers of dietary material (Plate 4:E).
0.5	chloroform	>30	sphericle	<60	Thick walls, cross- linking permeating right through particle (Plate 4:F; Plate 5:A and B).
3.0	chloroform	10-16	sphericle	<80	Smooth non-granular strong walled microcapsules (Plate 5:C and D).

Table 4. Effect of different acid chlorides and solvent systems on microcapsule construction.

Acid chloride	Solvent system (25 ml)	Wall thickness (um)	Shape	Particle size (um)	Wall texture and description of microcapsule.
Succinyl dichloride (0.1 ml)	Cyclohexane	10-30	sphericle	60-85	Smooth thick walls with bulk of diet incorporated into wall structure (Plate 5:E and F)
"	Chloroform	4-8	sphericle	150+	Smooth thin relatively fragile walls (Plate 6:A and B).
Malonyl chloride (0.1 ml)	Cyclohexane	5-10	sphericle	<80	Strong walled microcapsules with wide range of size (Plate 6: C,D and E).
"	Chloroform	7-9	ovoid	>200	Flake attachments cover cracked walls (Plate 6:F; Plate 7:A).

Oxayl chloride (0.1 ml)	Cyclohexane	8-10	flattened sphere	>200	Weak wrinkled collapsed walls (Plate 7:B and C).
"	Chloroform	4-8	sphericle	>200	Thin walled micro- capsules with flake like internal, easily ruptured (Plate 7: D,E and F).

Table 5. Empiracle formulae, molecular weight and structure of acid chlorides used in the formation of microcapsules, in present experiment.

Acid chloride	Empiracle formula	Molecular weight	Structure
Oxayl chloride	$C_2O_2Cl_2$	126.93	$\begin{array}{c} O=C-Cl \\   \\ O=C-Cl \end{array}$
Malonyl chloride	$C_3H_2O_2Cl_2$	140.95	$\begin{array}{c} O=C-CH_2-C=O \\   \qquad   \\ Cl \qquad Cl \end{array}$
Succinyl dichloride	$C_4H_4O_2Cl_2$	154.99	$\begin{array}{c} O \qquad O \\    \qquad    \\ Cl-C-CH_2-CH_2-C-Cl \end{array}$
Sebacoyl chloride	$C_{10}H_{16}O_2Cl_2$	239.14	$\begin{array}{c} O=C-(CH_2)_8-C=O \\   \qquad   \\ Cl \qquad Cl \end{array}$

Table 6a. Mean pH of seawater with different artificial diets. 100 mg of diet of <20 μm in 200 ml of U.V irradiated 0.2 μm cartridge filtered seawater.

Time (h)	Artificial diets				
	Microencapsulated		Microparticulate*1		Control
	AR103	AR118 AR120	HbK*2	Carageenan	Zein (no diet)
0	7.02	6.29	6.30	7.46	7.55
24	7.95	7.73	7.63	7.96	7.49
48	7.88	7.83	7.85	7.93	8.03
72	7.88	7.86	7.83	8.04	7.35
					7.50
					7.42
					7.99
					7.31
					8.02
					8.04
					7.98
					8.03

\*1 Kanazawa et al.,1971, 1982.  
 \*2 Microencapsulated haemoglobin and Kanazawa et al.,1971 diet B .

Table 6b. Mean pH of distilled water with artificial diets, concentration as above.

Time (h)	Artificial diets				
	Microencapsulated		Microparticulate*1		Control
	AR118	AR120 CD227	CD227#	HbK HbK#	Carageenan (no diet)
0	3.53	3.68	3.85	5.12	4.13
24	3.49	3.57	3.86	5.06	4.28
48	3.53	3.57	3.81	5.02	4.38
72	3.55	3.51	3.83	5.10	4.05
					5.32
					5.14
					5.25
					5.37
					6.68
					6.43
					6.01
					5.72
					5.60
					5.63
					5.58
					5.61

\*1 Kanazawa et al.,1971, 1982.  
 # pre-washed for 15 minutes.

Table 7a. Mean percentage rate of release of water soluble protein from microencapsulated and microparticulate diets. 100 mg of diet in 200 ml of U.V. irradiated 0.2µm cartridge filtered seawater.

Artificial diet	Time (h)		Total available soluble <sub>1</sub> protein (µg ml <sup>-1</sup> )
	2	24      48	
Microencapsulated			
CD136	10.25	28.20   81.25	19.30
CD301	4.62	16.20   41.46	17.62
CD434	3.85	17.34   42.38	18.44
Microparticulate*1			
Carageenan bound	18.07	66.26   84.35	33.37
Zein bound	34.14	58.53   87.80	16.48

\*1 Kanazawa et al.,1971,1982.

Table 7b. Presence (+) of bacterial growth and protozoa in seawater after 24 and 48 h, following the addition of artificial diets. Concentration and conditions as above.

Artificial diet	Smell	Formation of clumps	Bacterial attachment to diet	Bacteria in water column	Presence of protozoa
Control (no diet)					
24 h	-	-	-	-	-
48 h	-	-	-	-	-
Microencapsulated diets.					
CD136	-	+	-	+	-
	-	+	+	+	+
CD301	-	+	-	-	-
	-	+	-	+	-
CD434	-	-	-	-	-
	-	+	-	+	-
Microparticulate diets*1					
Carageenan bound diet	+	+	+	+	-
	+	+	+	+	+
Zein bound diet	+	+	+	+	-
	+	+	+	+	+

Commercially  
available  
microparticulate  
diets.

Nippai

Kyowa-  
Hakko

+	+	-	+	+	-
+	+	+	+	+	+
+	+	+	+	+	-
+	+	+	+	+	+

\*1 Kanazawa et al.,1971,1982.

Table 8. Time (seconds) for methylene blue dye from four sites, to reach an MF aerator, raised to different heights off the pool floor, situated in the centre of a pool (3.66m diameter and 0.91m deep).

Height of MF aerator off the pool floor. (cm)	Site			
	A	B	C	D
4	i 45	60	90	60
	ii 35	65	60	70
10	i 55	90	-	70
	ii 45	100	80	60
25	i 120	150	-	100
	ii 100	-	70	90
50	i -	-	-	-
	ii -	-	-	-

i = dye released just below the surface.  
ii= dye released at depth.  
- = greater than 15 minutes.

Figure 1. Procedure for the preparation of cross-linked protein microcapsules, (British patent Nos 79437454).

Emulsification of the amine.

5ml diet: 20% w/v Haemoglobin sieved to <20 $\mu$ m, made up in distilled water.	----->	<-----25ml cyclohexane  <-----1ml Span 85 (Sorbitan trioleate)
150ml beaker		
5 min stir at 200 r.p.m with 3cm magnetic follower.		

Interfacial polymerisation reaction.

		<-----10ml cyclohexane with 1% v/v Succinyl dichloride.
Reaction allowed to continue for 15 min.		
25ml cyclohexane (dropwise)	----->	
30ml cyclohexane	----->	
Allow particles to settle and discard supernatant.		

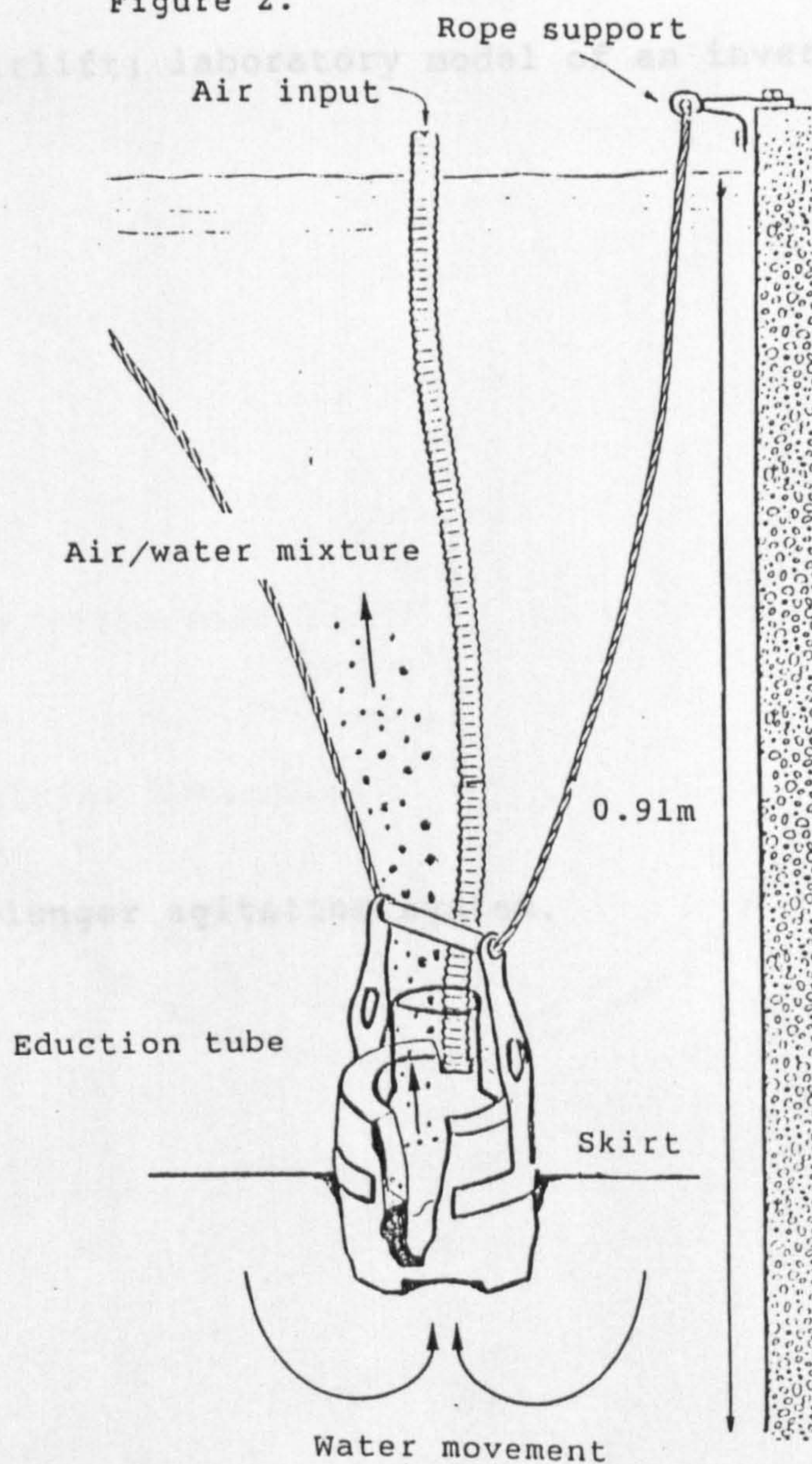
Isolation of microcapsules.

		<-----50ml cyclohexane with 2% w/v lecithin.
5 min stir		
Allow particles to settle and discard supernatant.		
15% w/v gelatin solution made up in distilled water.	----->	
Rinse in warm water and lyophilise.		

Figure 2. MF aerator (Venturator Ltd). Designed for rapid vertical lift of air/water mixture, supplied with 15 c.f.m.

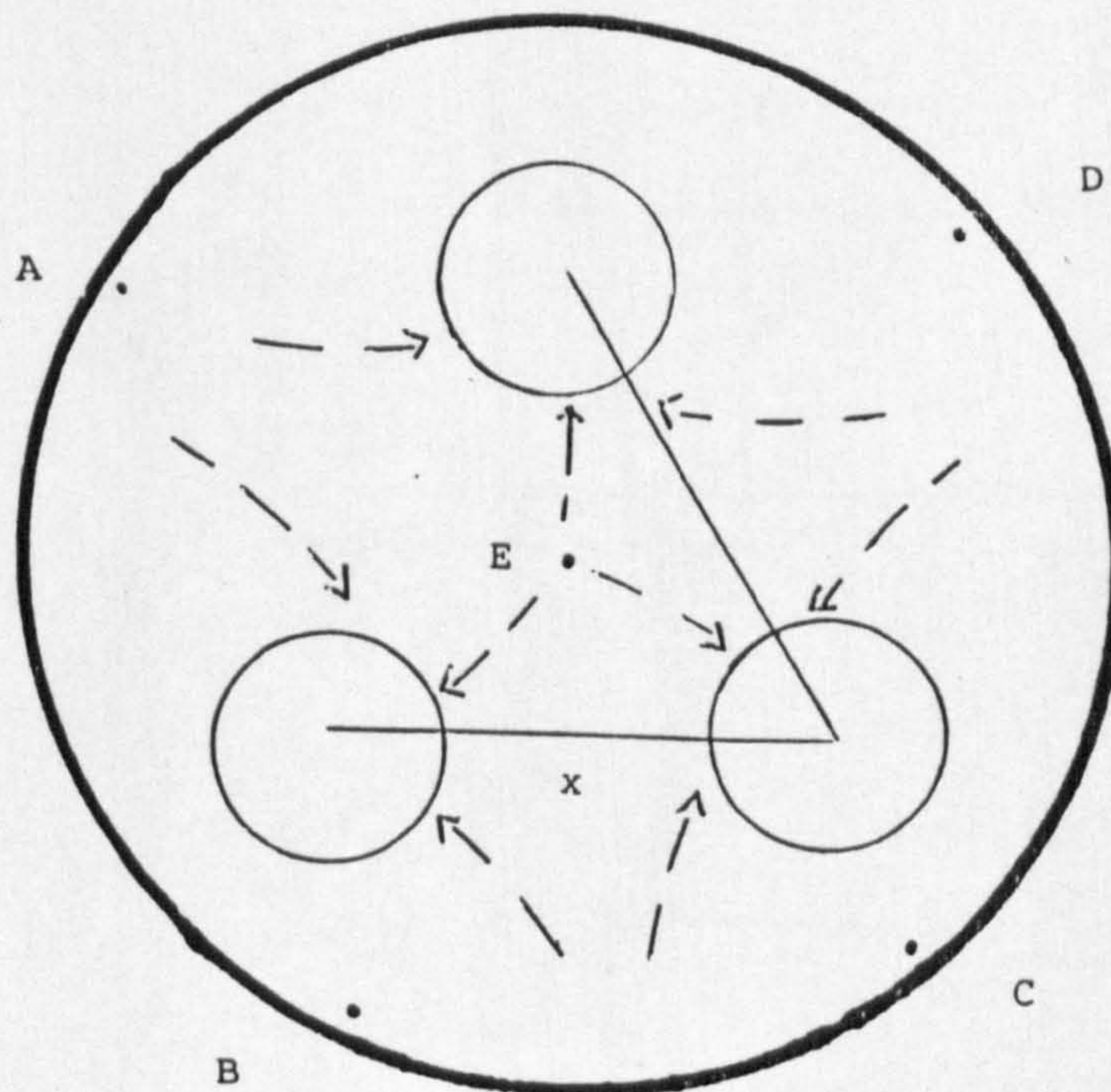
Figure 3. Sites A-E of aliquot sample removal to determine percentage particles remaining in suspension with time and dye patterns produced as a result of the MF aerators.

Figure 2.



→  
Dye pathway

Figure 3.



$x = 116\text{cm}$

**Figure 4. Airlift; laboratory model of an inverted plastic pipette.**

**Figure 5. Plunger agitation system.**

Figure 4.

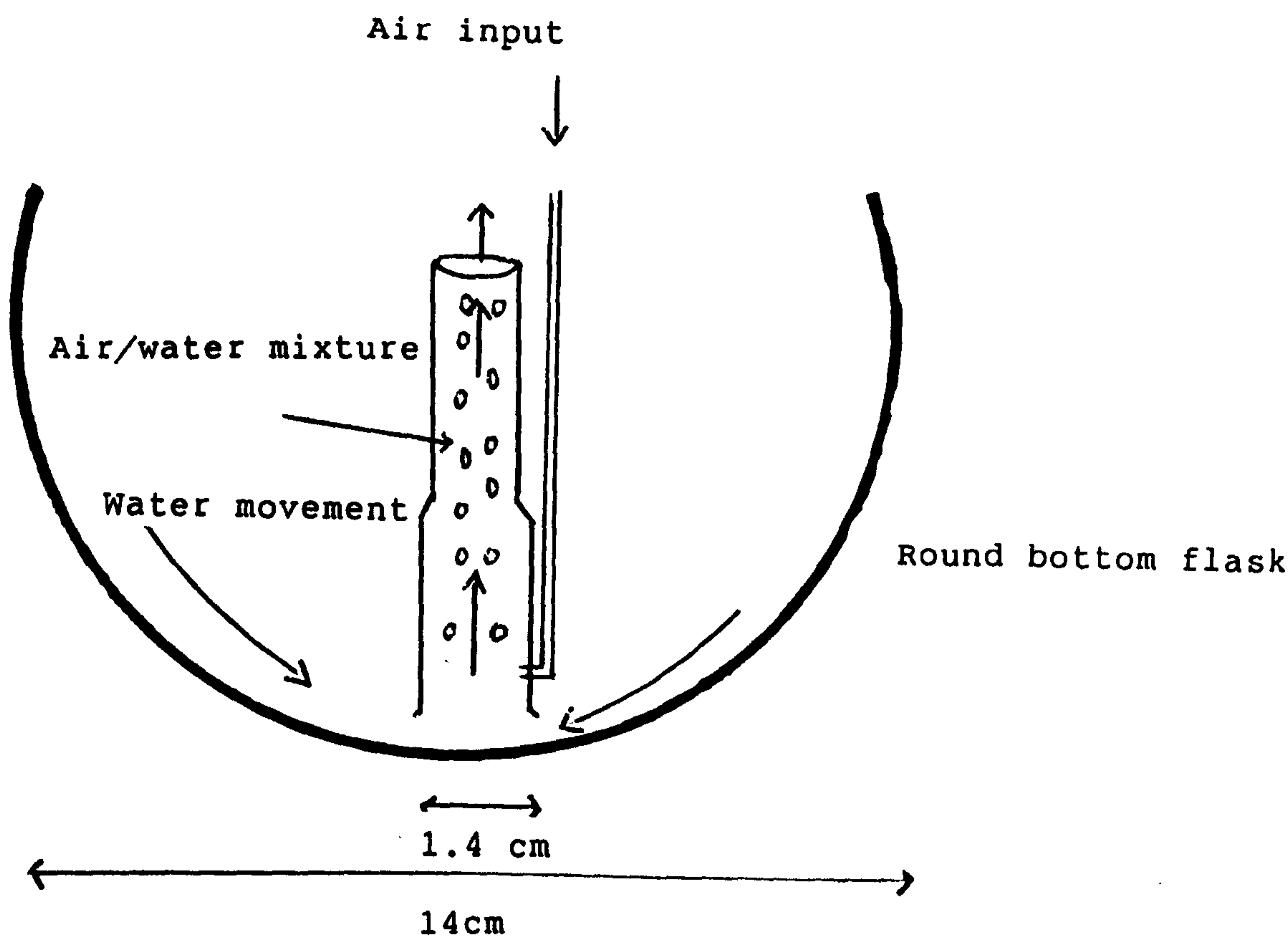


Figure 5.

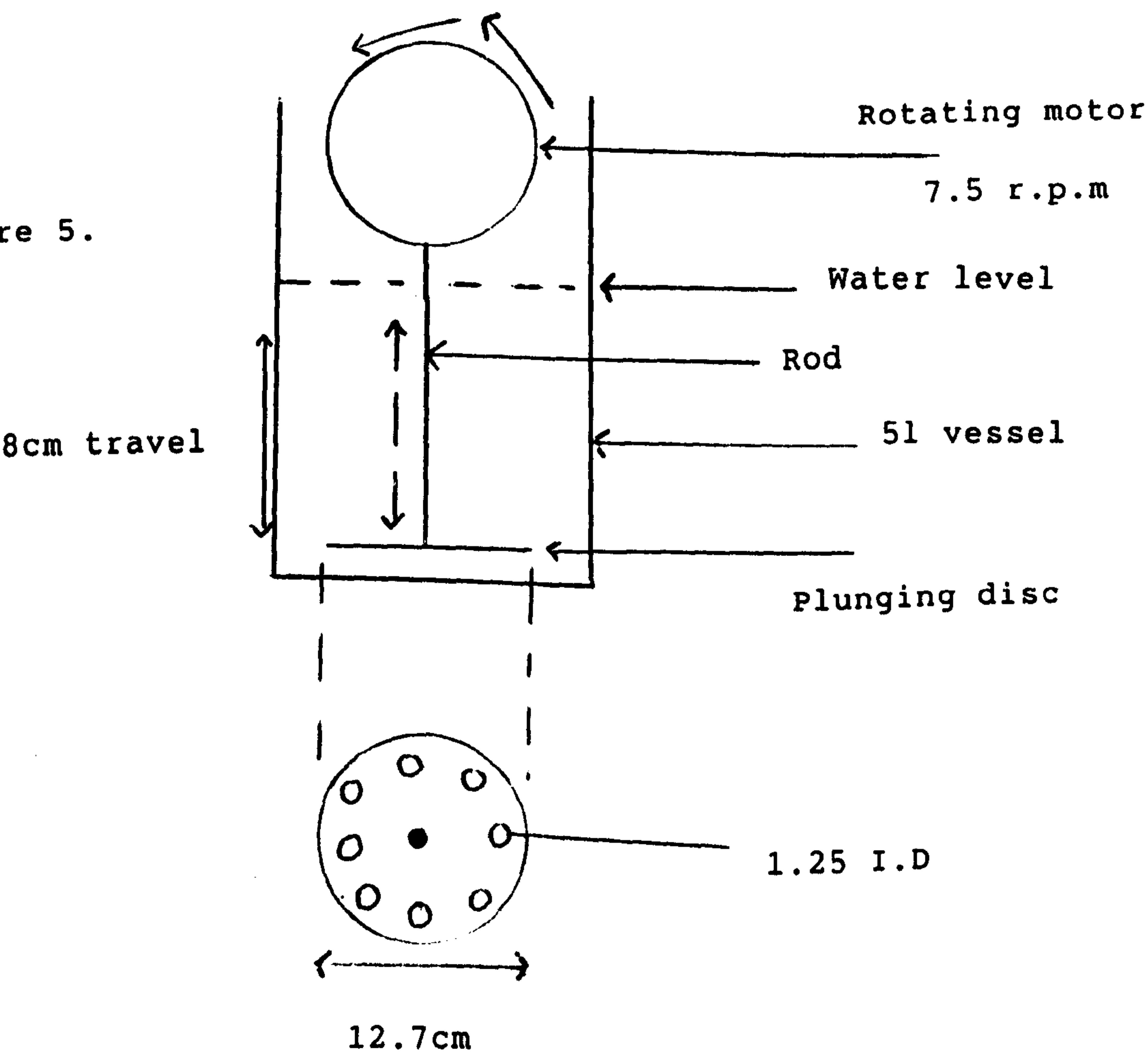


Figure 6a. Mean pH of seawater containing artificial diets. 100mg of diet of  $< 20 \mu\text{m}$  in 200 ml of U.V irradiated  $0.2 \mu\text{m}$  cartridge filtered seawater. Control seawater= no artificial diet.

Microencapsulated diets;

A= AR103

B= AR118

C= AR120

D= Microencapsulated haemoglobin and Kanazawa et al.,(1971) diet B.

Microparticulate diets;

E= Kanazawa et al.,(1971,1982) diet B bound in carageenan.

F= Kanazawa et al.,(1971,1982) diet B bound in zein.

G= control.

Figure 6b. Mean pH of distilled water containing artificial diets. 100mg of diet of  $< 20 \mu\text{m}$  in 200 ml of U.V irradiated  $0.2 \mu\text{m}$  cartridge filtered seawater. Control distilled water= no artificial diet.

Microencapsulated diets;

A= AR118

B= AR120

C= CD227

D= CD227 pre-washed for 15 minutes.

E= Microencapsulated haemoglobin and Kanazawa et al.,(1971) diet B.

F= Microencapsulated haemoglobin and Kanazawa et al.,(1971) diet B pre-washed for 15 minutes.

Microparticulate diet;

G= Kanazawa et al.,(1971,1982) diet B bound in carageenan.

H= control.

Figure 6a

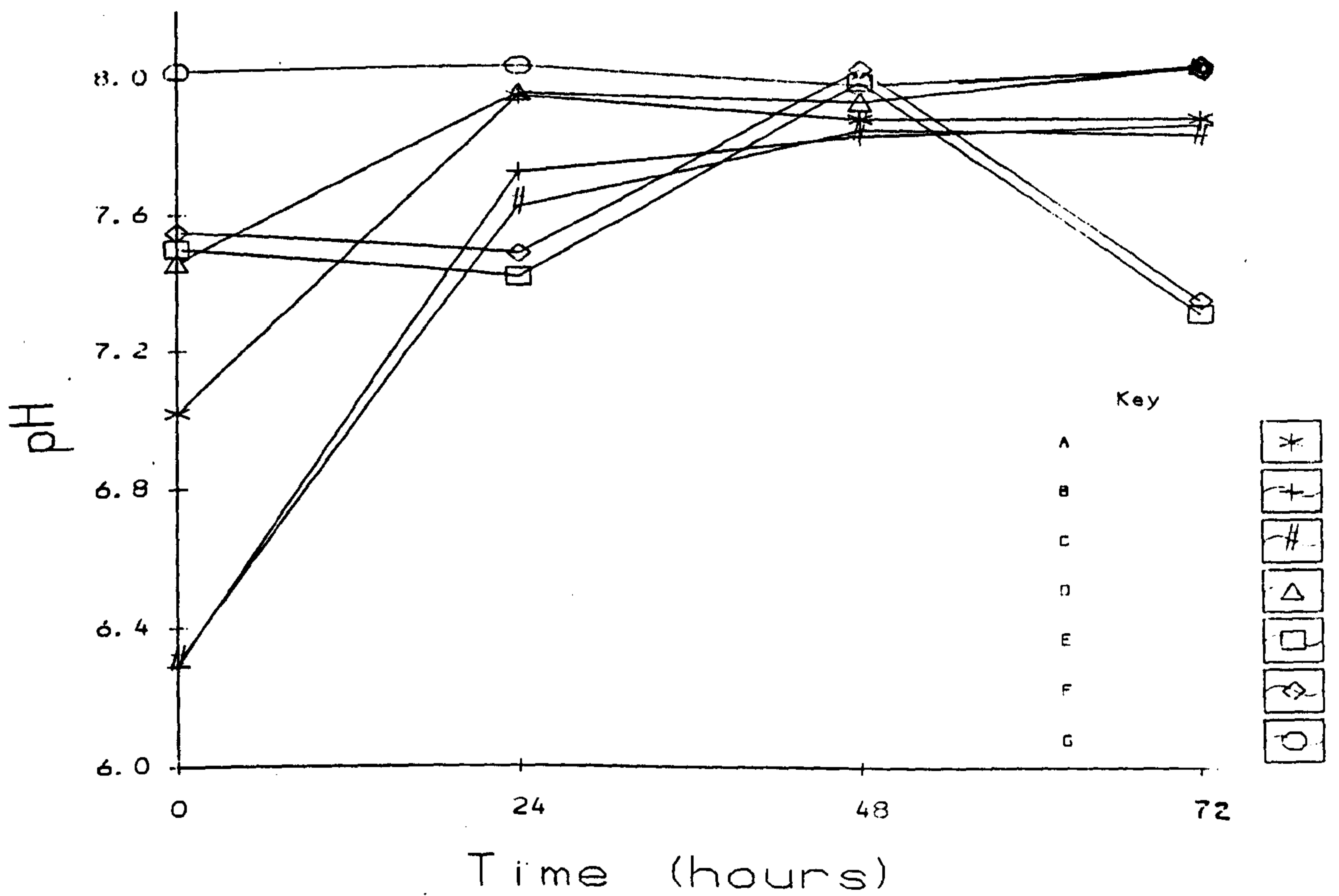


Figure 6b

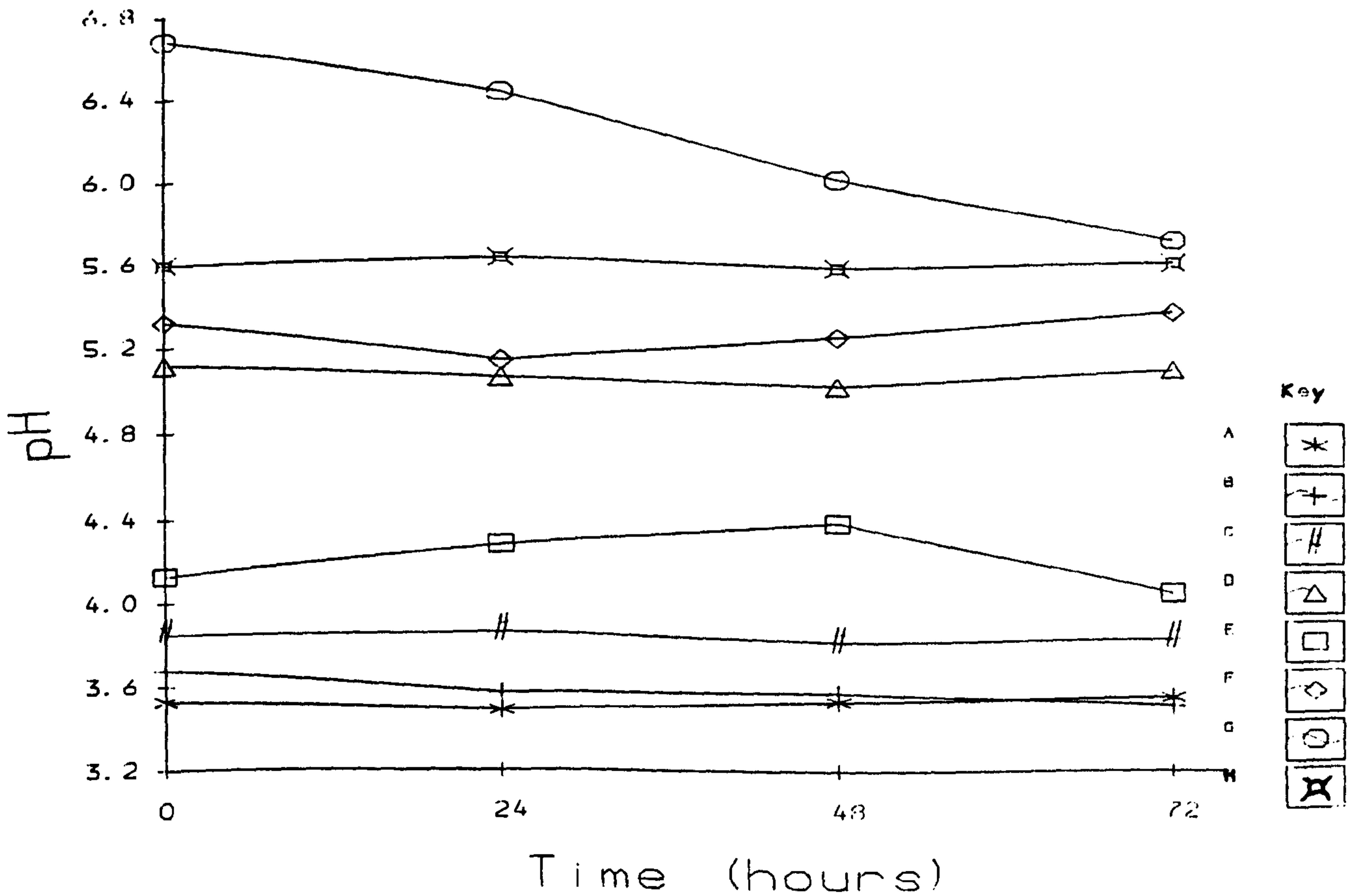


Figure 7a. Mean ln % of particles remaining in suspension. 30g of microencapsulated diet in 9620.9l (Polar Pools Ltd) of seawater with no agitation. Temperature= 15°C

Code	Diet	Regression line	Coeff of determination
A=	AR114 <80 µm	ln Y= 4.89+ (-0.049xX)	0.983
B=	CD136 60-90 µm	ln Y= 4.87+ (-0.053xX)	0.989
C=	CD136 90-150 µm	ln Y= 4.76+ (-0.054xX)	0.981

At 13 degrees of freedom the F value =0.32 and indicates no significant difference between the regression lines at the 5% level.

Figure 7b. Mean ln % of particles remaining in suspension. 30g of microencapsulated diet in 9620.9 l (Polar Pools Ltd) of seawater with continuous (Diet codes A,B and C) and discontinuous agitation( Diet codes D,E and F), provided by MF Ventura aerators. Temperature= 15°C

Code	Diet	Regression line	Coeff of determination
A=	AR114 <30 µm	ln Y= 4.70+ (-0.304xX)	0.979
B=	CD136 60-90 µm	ln Y= 4.87+ (-0.379xX)	0.957
C=	CD136 90-150 µm	ln Y= 4.55+ (-0.402xX)	0.968
D=	AR114 <30 µm	ln Y= 4.45+ (-0.187xX)	0.982
E=	CD136 60-90 µm	ln Y= 4.40+ (-0.193xX)	0.940
F=	CD136 90-150 µm	ln Y= 4.48+ (-0.190xX)	0.968

Between 6 to 10h considerably more particles are found in suspension with the discontinuous agitation system. df=17; F=11.58; P=0.004.

FIGURE 7A

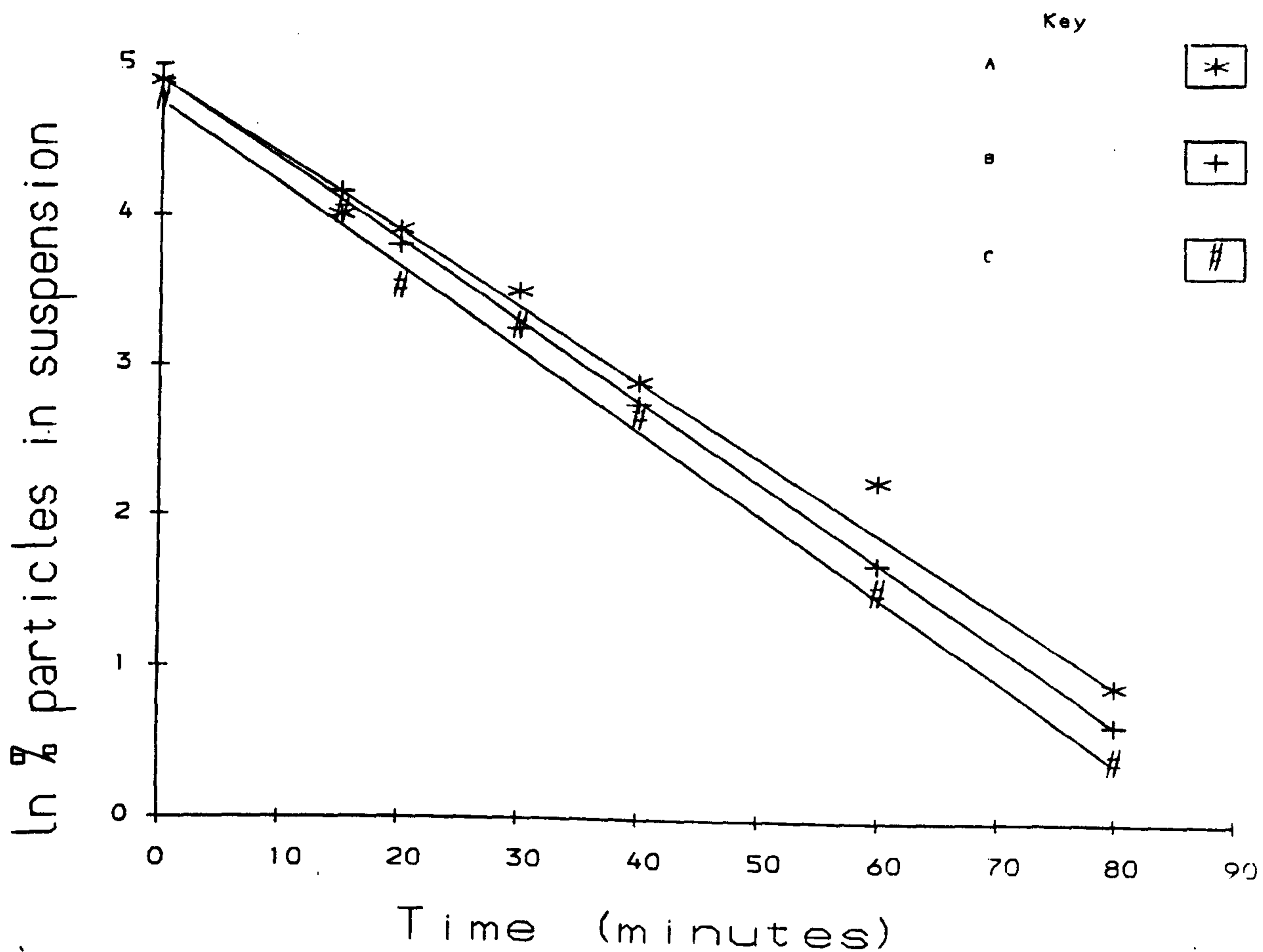


FIGURE 7B

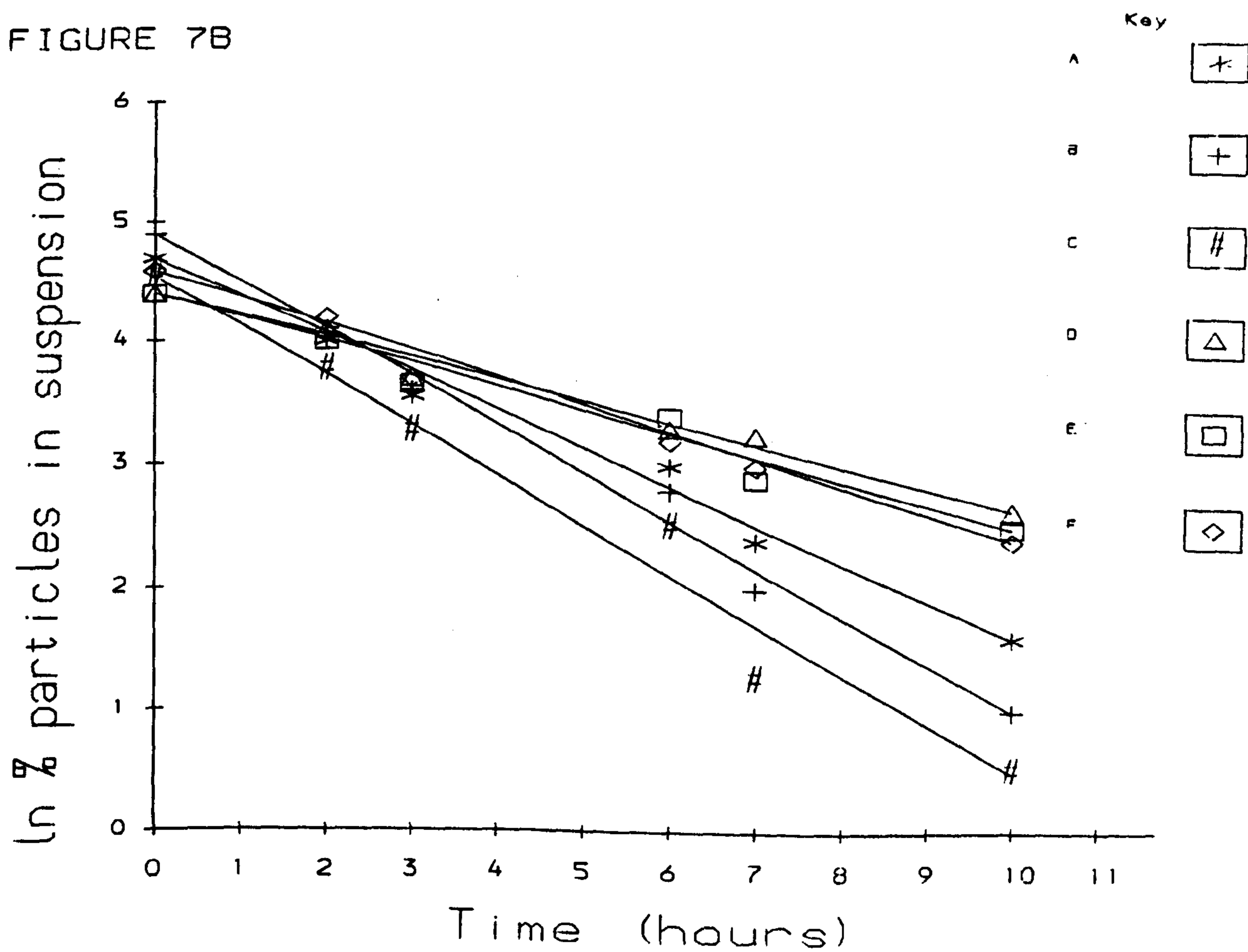


Figure 8. Mean % particle size distribution of artificial diets suspended in U.V irradiated 0.2  $\mu$ m cartridge filtered seawater, after 15 minutes. 10mg of diet in 200ml of seawater. Temperature= 20<sup>0</sup>C; No agitation.

Code Diet.

A= Kyowa-Hakko commercial diet.

B= Charcoal powdered.

C= CAR003 (Frippak Feeds).

D= Nippai commercial diet.

Figure 9. Mean ln % particles remaining in suspension in 2l round-bottom flasks filled with U.V irradiated 0.2  $\mu$ m cartridge filtered seawater. Only regression lines shown. Concentration = 20mg in 2l of seawater. Temperature= 20<sup>0</sup> C, with no agitation.

Code	Diet	Regression line	Coeff of determination
A=	Charcoal	$\ln Y=4.55+(-0.0051xX)$	0.963
B=	Haemoglobin	$\ln Y=4.60+(-0.0044xX)$	0.996
C=	AR103	$\ln Y=4.60+(-0.0069xX)$	0.993
D=	CAR003	$\ln Y=4.63+(-0.0027xX)$	0.993
E=	Nippai	$\ln Y=4.62+(-0.0072xX)$	0.968
F=	Kyowa-Hakko	$\ln Y=4.60+(-0.0081xX)$	0.990

Figure 9. Continued. Pairwise comparison on the settlement rate ( $\ln$  % particles in suspension  $\text{min}^{-1}$ ) derived from linear regression analysis.  $SE^2 = 2.1 \times 10^{-7}$ ;  $Q(0.05, 6, 36) = 4.265$ ;  $M.S.D = 1.96 \times 10^{-3}$ .

Summary of the significance using Tukey's test.

CAR003 Haemoglobin Charcoal AR103 Nippai Kyowa-Hakko

CAR003

Haemoglobin	n.s					
Charcoal	*	n.s				
AR103	*	*	n.s			
Nippai	*	*	*	n.s		
Kyowa-Hakko	*	*	*	*	n.s	

A \* denotes significance at 5% level, n.s denotes no significant difference.

FIGURE 8

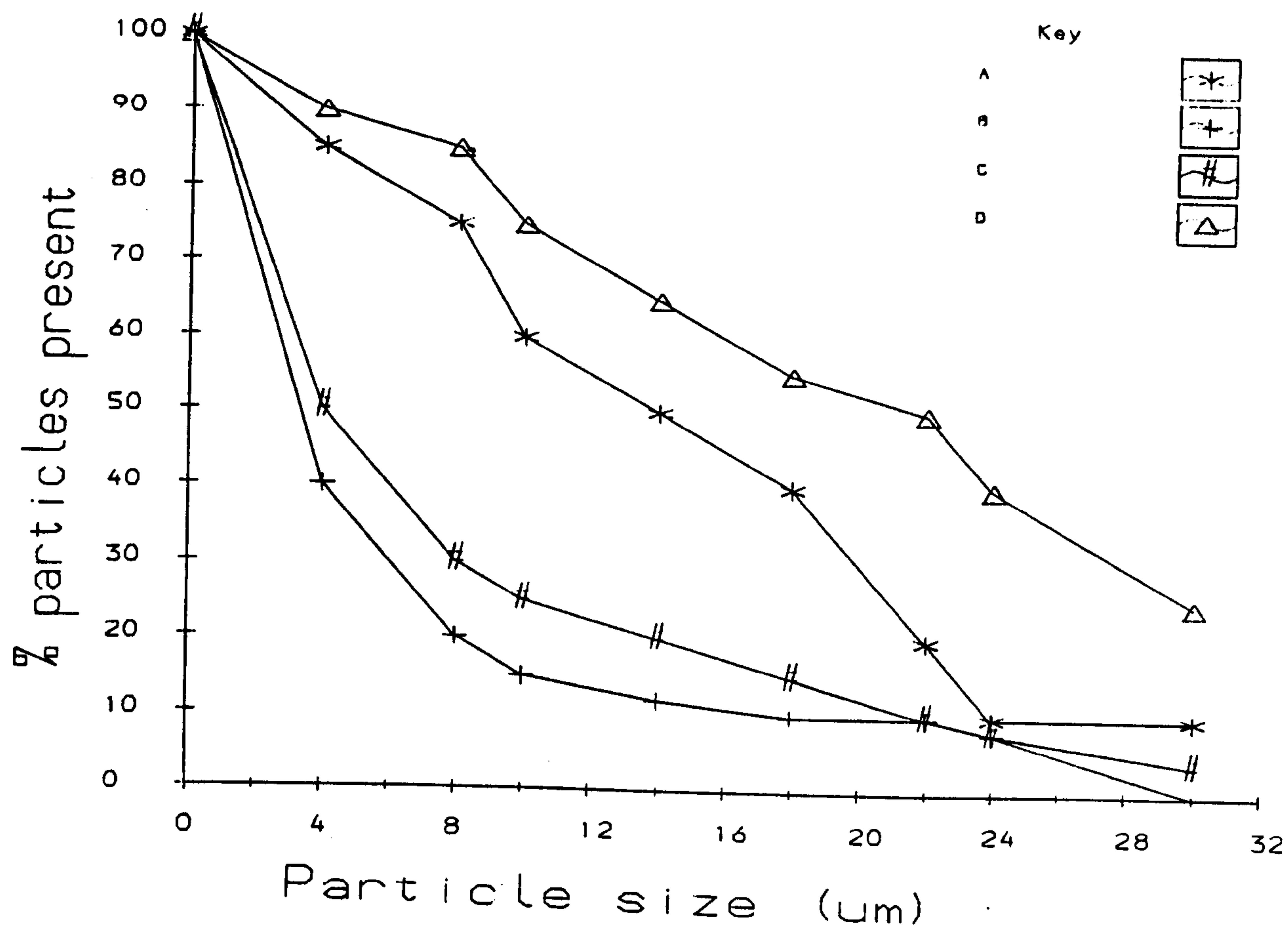


FIGURE 9

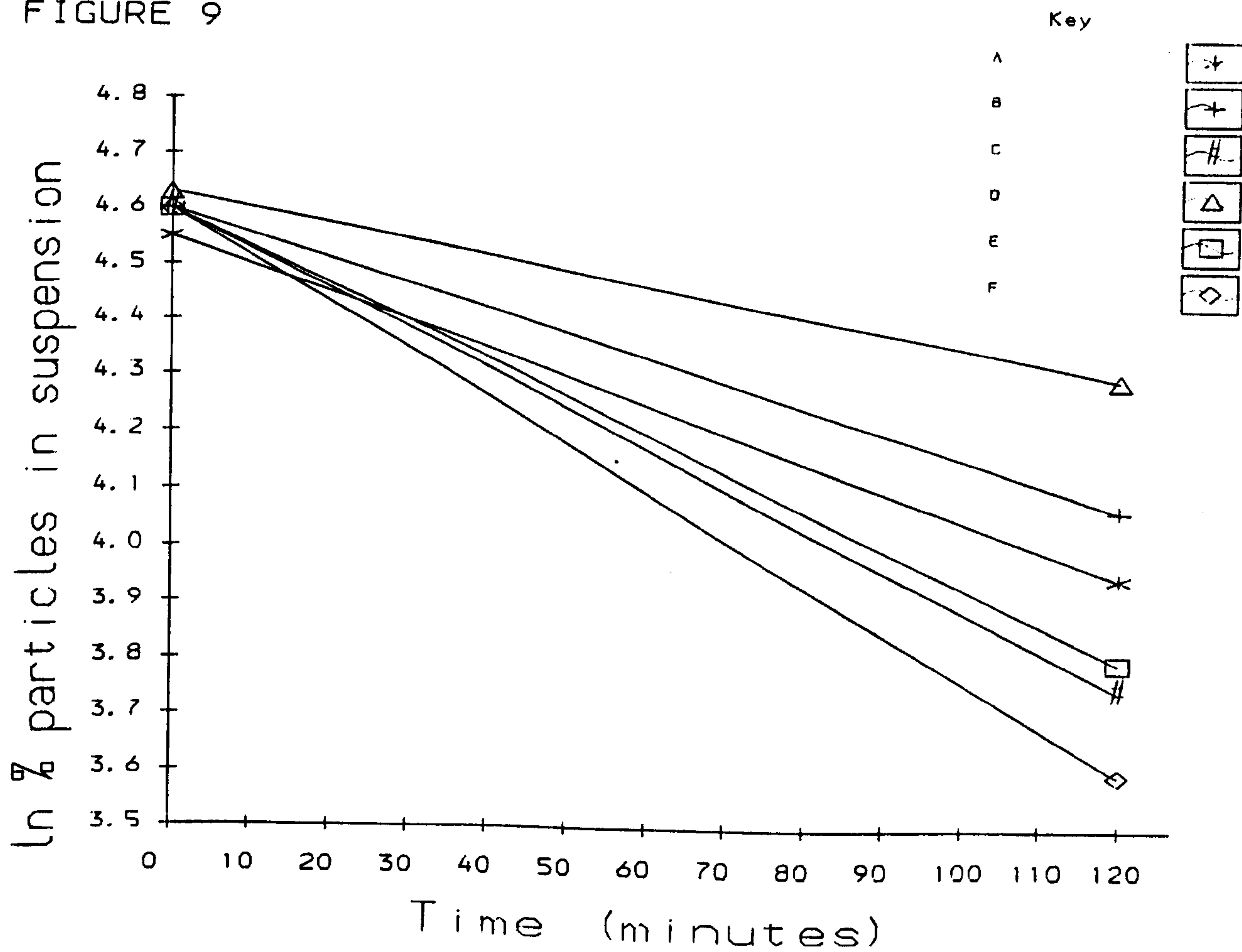


Figure 10. Mean ln % of microencapsulated diet CAR003 remaining in suspension in 2l round-bottom flasks filled with U.V irradiated 0.2  $\mu$ m filtered seawater. Unless otherwise stated each flask was fitted with an aeration system operating continuously. Concentration= 20mg in 2l. Temperature= 20°C. Only regression lines shown.

Code	Aeration system	Regression line	Coeff of determination
A=	Airstone	$\ln Y=4.24+(-0.073xX)$	0.987
B=	Pipette position flask side (1mm I.D)	$\ln Y=4.28+(-0.073xX)$	0.976
C=	Pipette position flask centre (1mm I.D)	$\ln Y=3.96+(-0.124xX)$	0.996
D=	Pipette position flask side (5mm I.D)	$\ln Y=4.37+(-0.007xX)$	0.995
E=	Pipette position flask centre (5mm I.D)	$\ln Y=4.28+(-0.008xX)$	0.977
F=	Pipette*1 position flask centre (5mm I.D)	$\ln Y=4.17+(-0.006xX)$	0.990

\*1 aeration 'ON' 5 minutes every 30 minutes.

Figure 11. Mean % of microencapsulated diet CAR003 remaining in suspension in 5l vessels filled with U.V irradiated 0.2  $\mu$ m filtered seawater, provided with agitation. Concentration= 20mg in 5l. Temperature= 20°C.

Code	Agitation system.
A=	Paddle stirrer: 'ON' 5 minutes every 30 minutes.
B=	Paddle stirrer: 'ON' 15 minutes every 30 minutes.
C=	Paddle stirrer: 'ON' continuously.
D=	Plunger system (figure 5): 'ON' continuously.
E=	Airlift (figure 4): 'ON' continuously.
F=	Pipette position flask side (5mm I.D): 'ON' continuously.

FIGURE 10

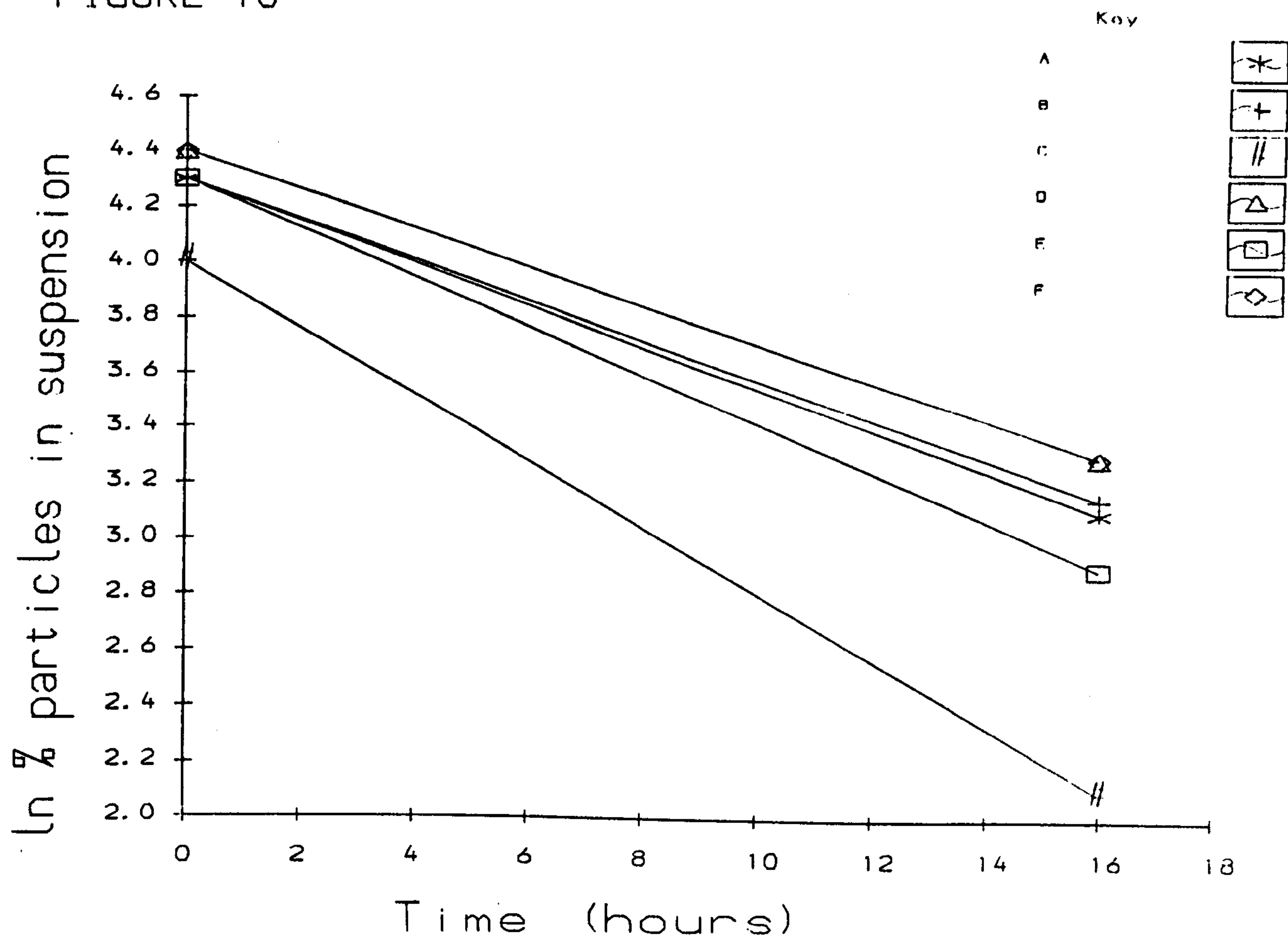


FIGURE 11

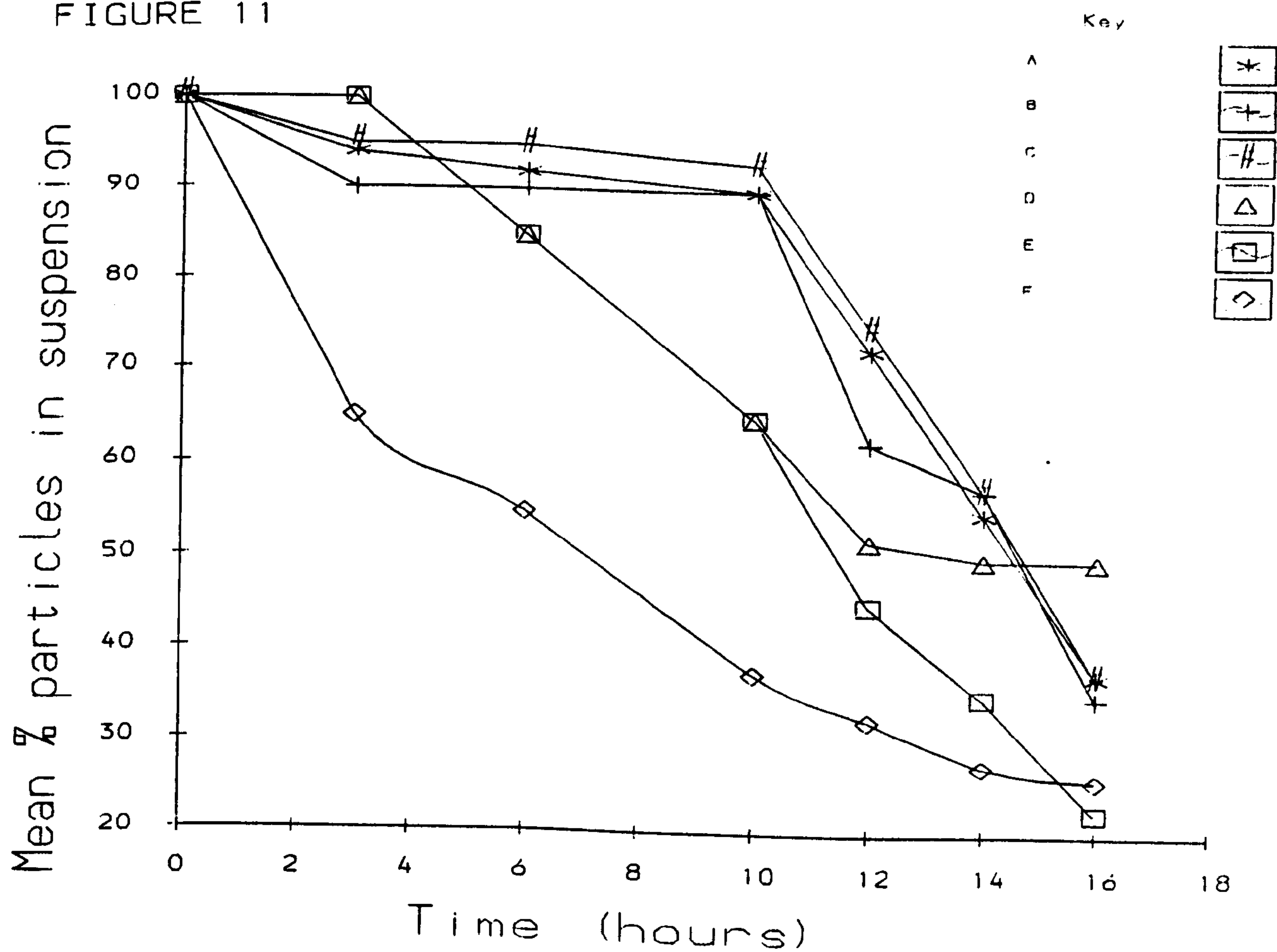
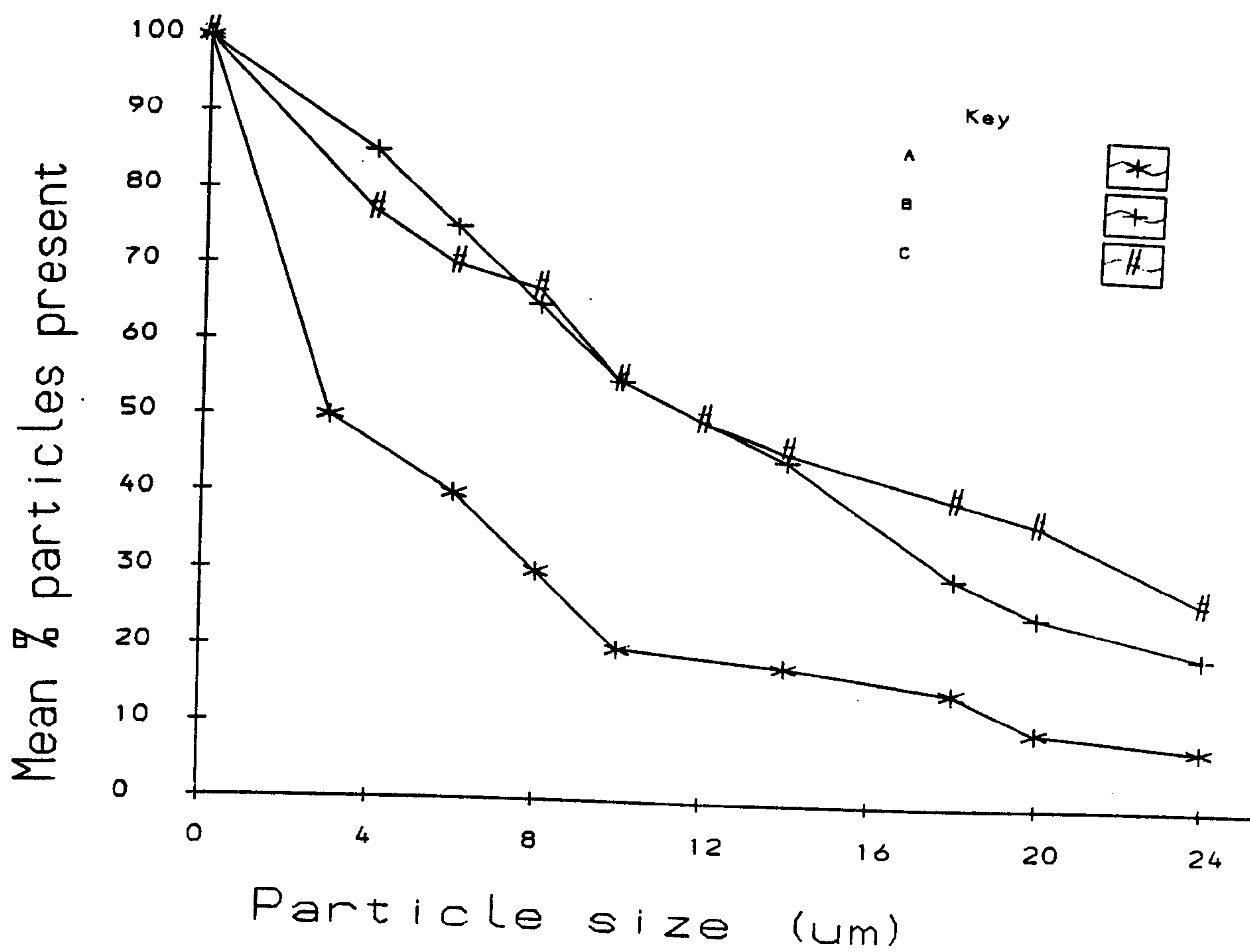


Figure 12. Mean % particle size distribution of microencapsulated diet CAR003 at T=10h, in 5l vessels filled with U.V irradiated 0.2  $\mu$ m filtered seawater, provided with agitation. Concentration= 20mg in 5l. Temperature= 20°C.

Code

- A= Control T=0h (no agitation).
- B= Plunger (figure 5), T=10h.
- C= Paddle stirrer, T=10h.

FIGURE 12



**Plate 1.**

**A) Piston pumps (6 p.s.i. and 15 c.f.m. each: B.V.C. Ltd).**

**B) Circular pool of volume 9621 l (3.66m diameter by 0.91m deep,  
PY 1236: Polar Pools Ltd).**

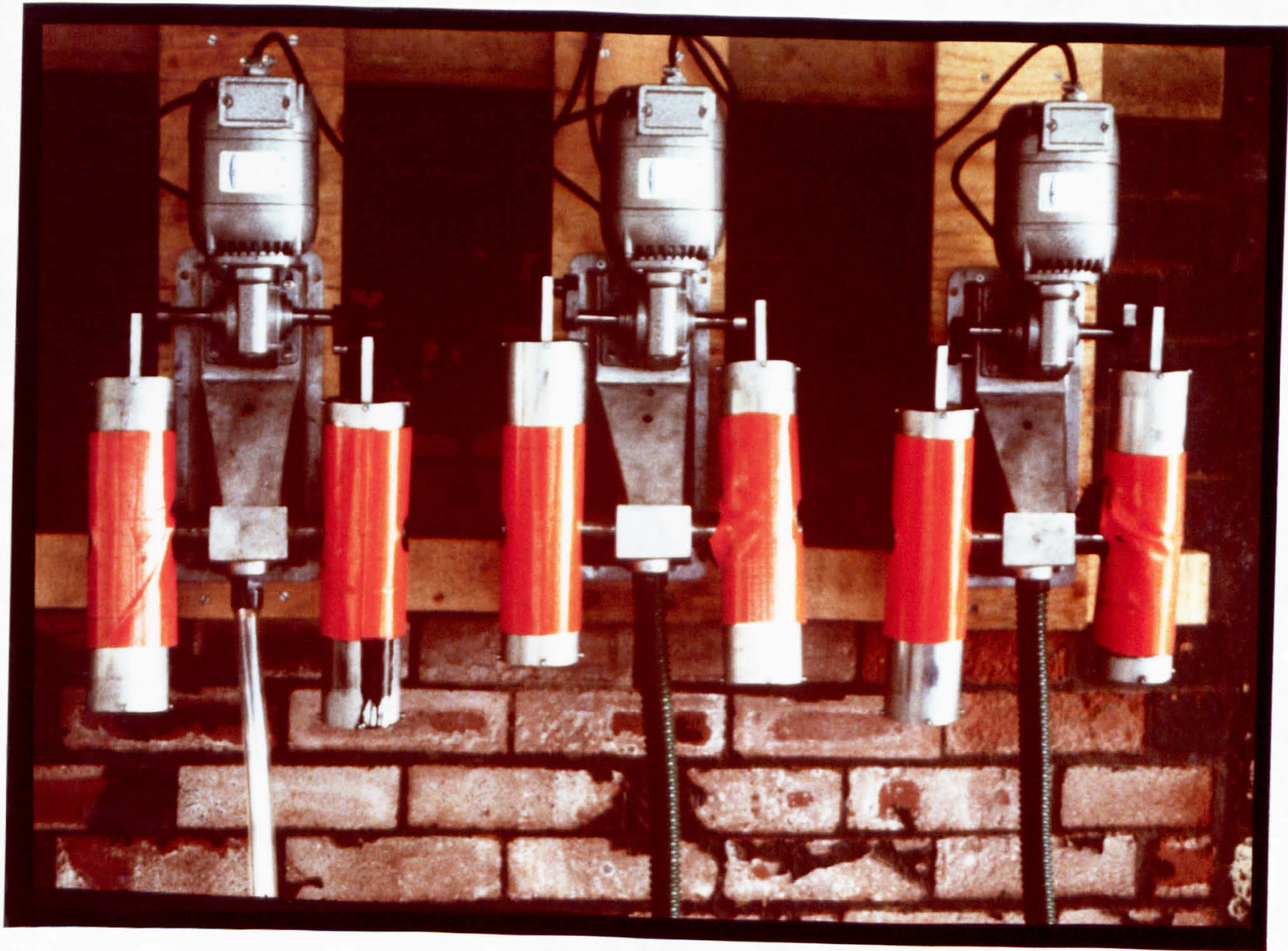


Plate 2.

A) Mag= x1500

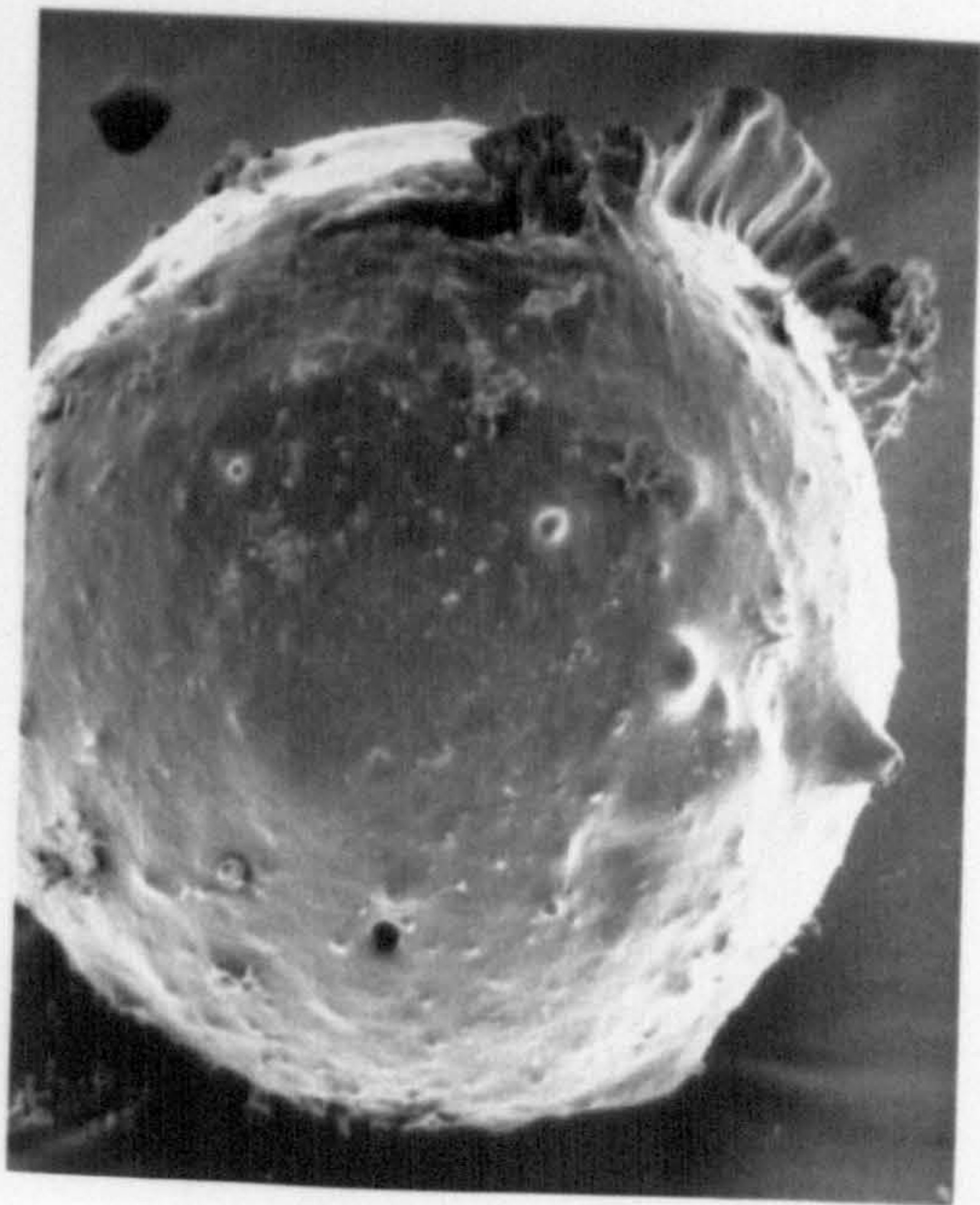
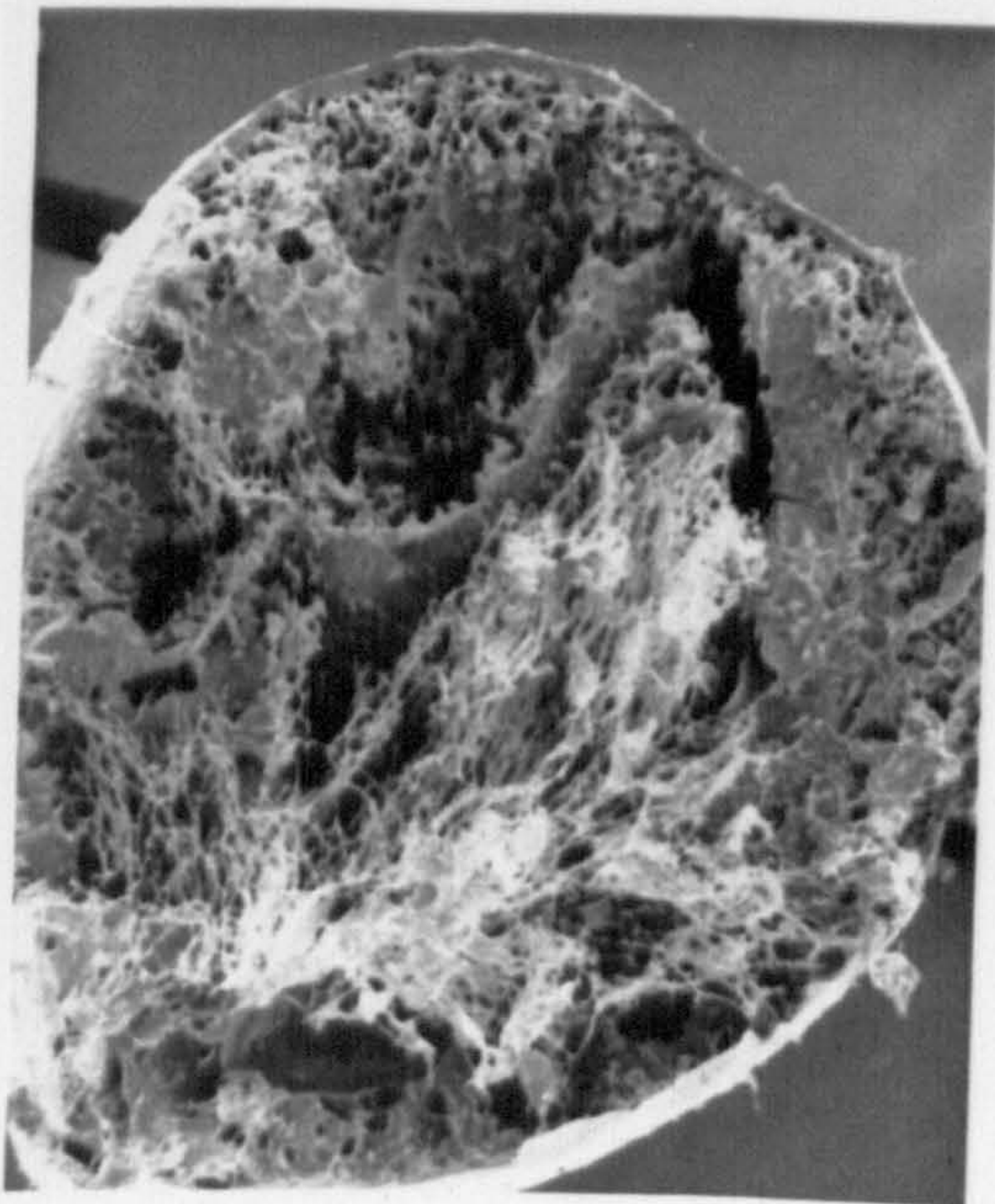
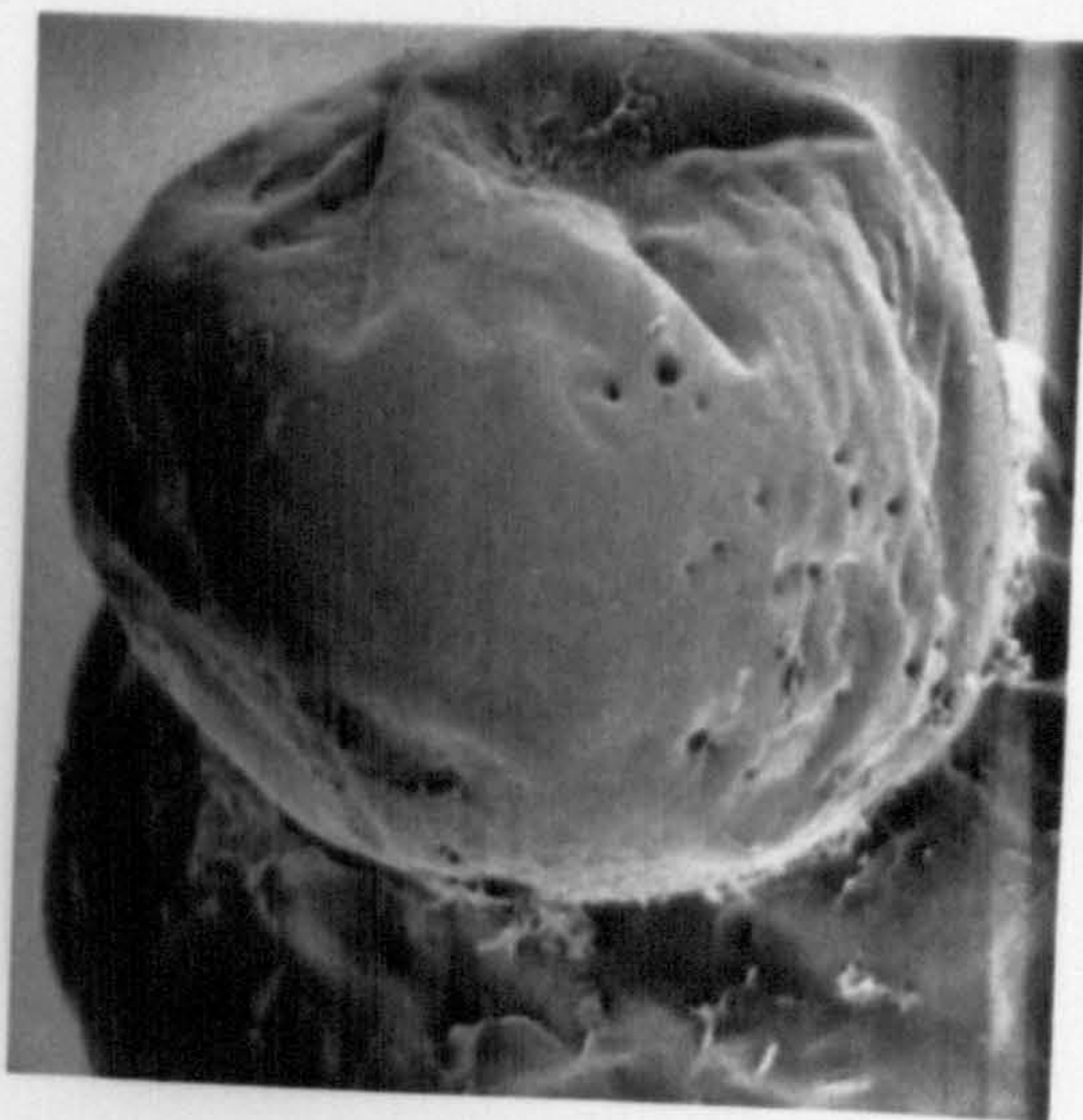
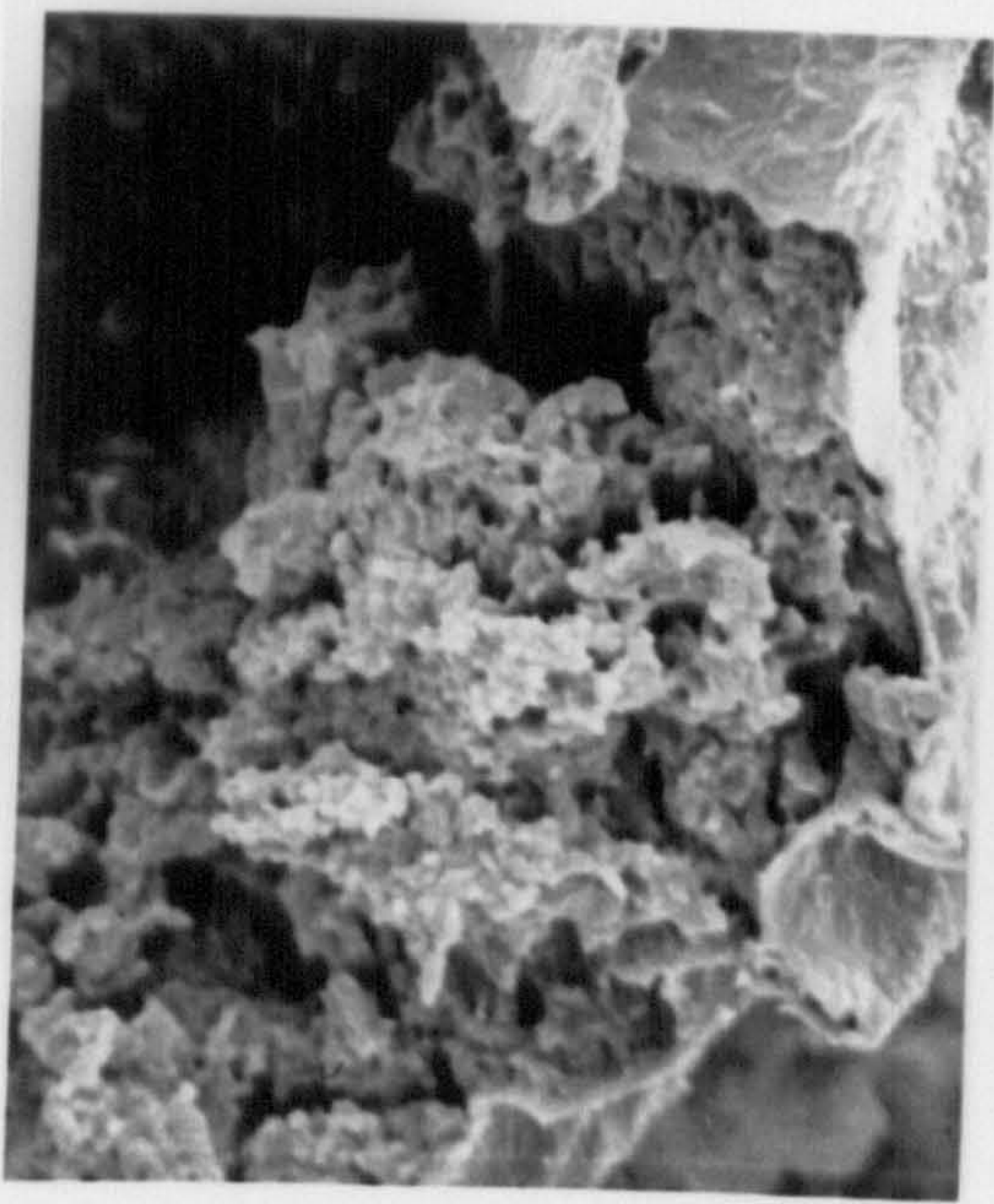
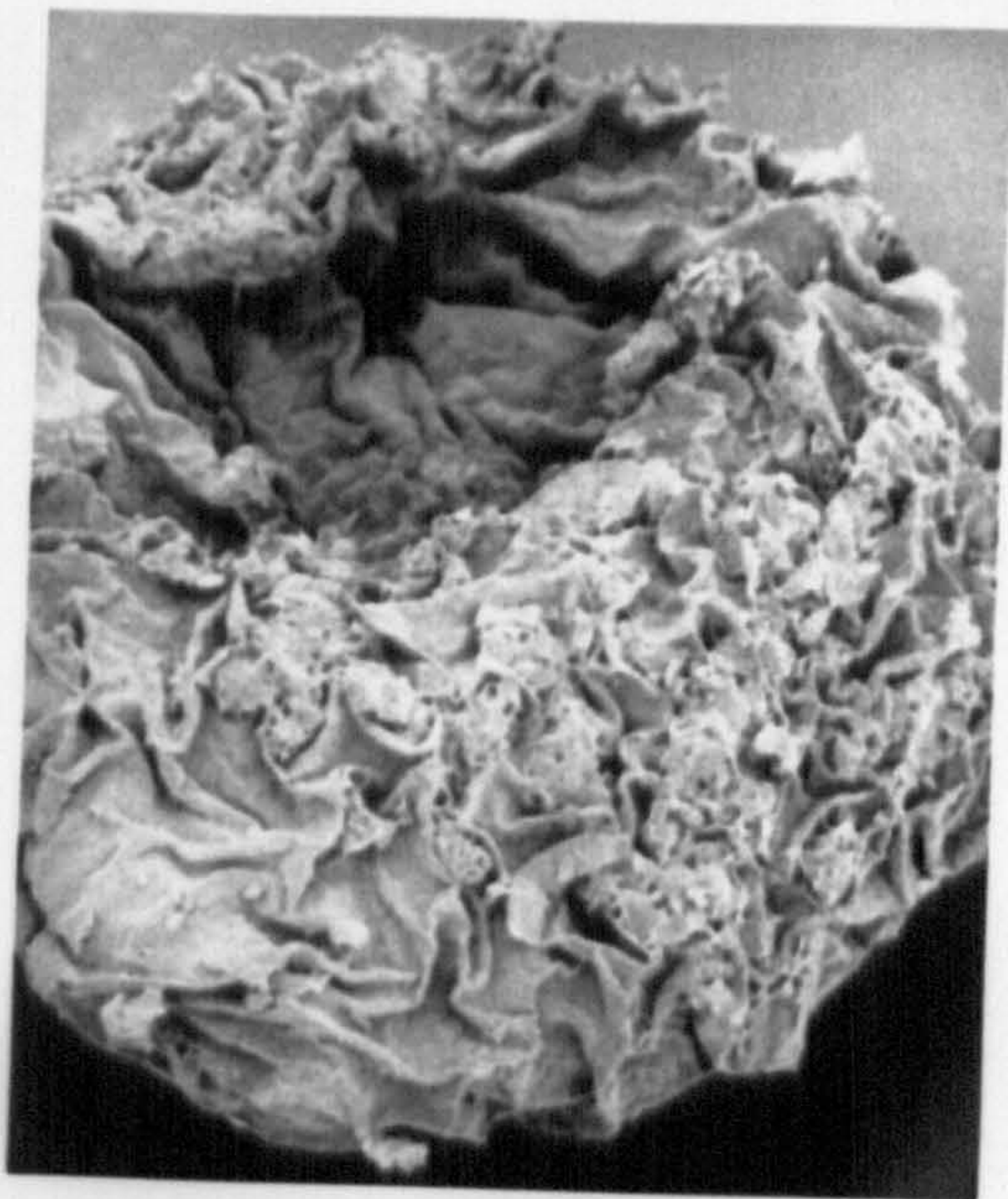
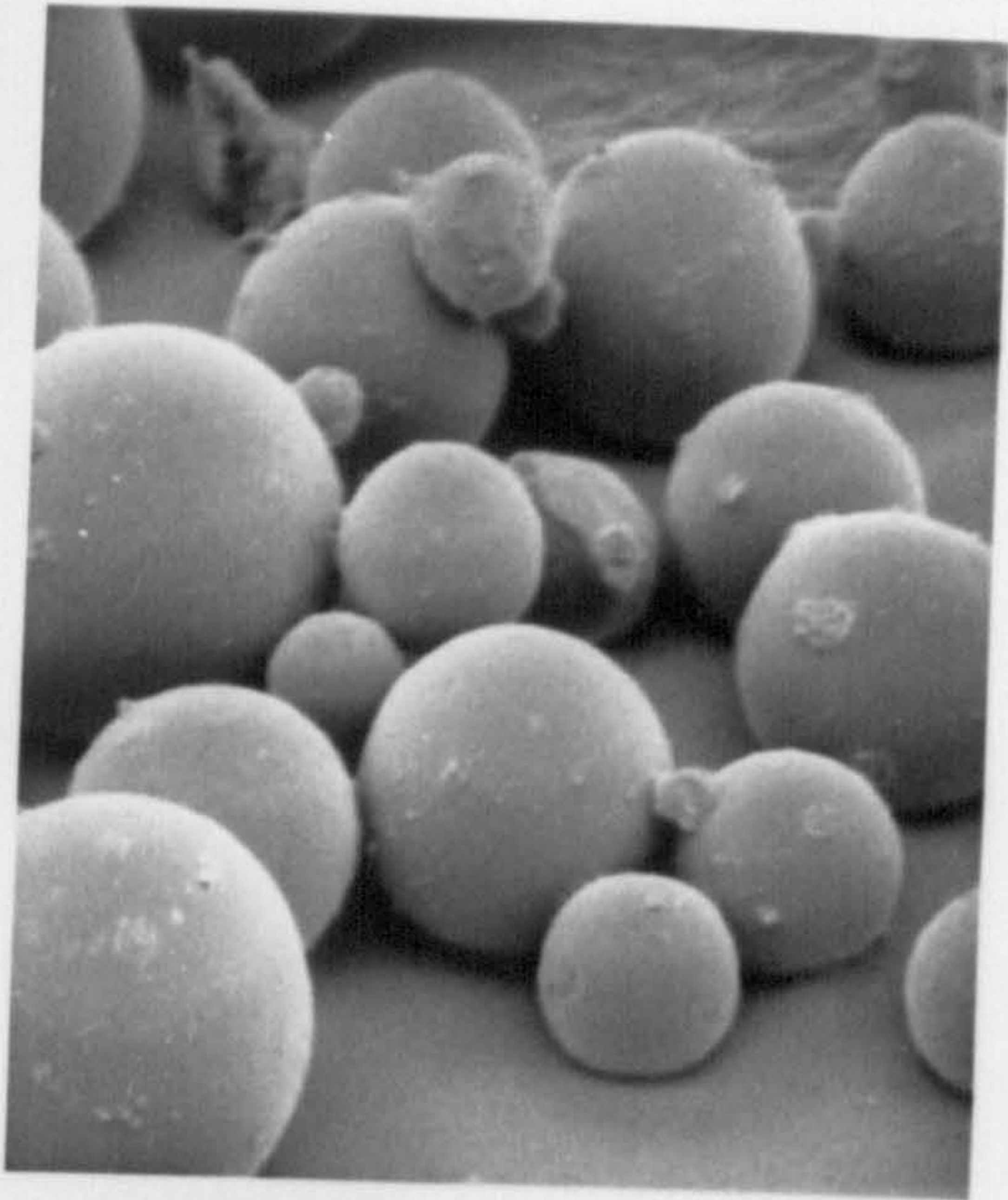
B) Mag= x220

C) Mag= x500

D) Mag= x800

E) Mag= x800

F) Mag= x100



**Plate 3.**

**A) Mag= x3000**

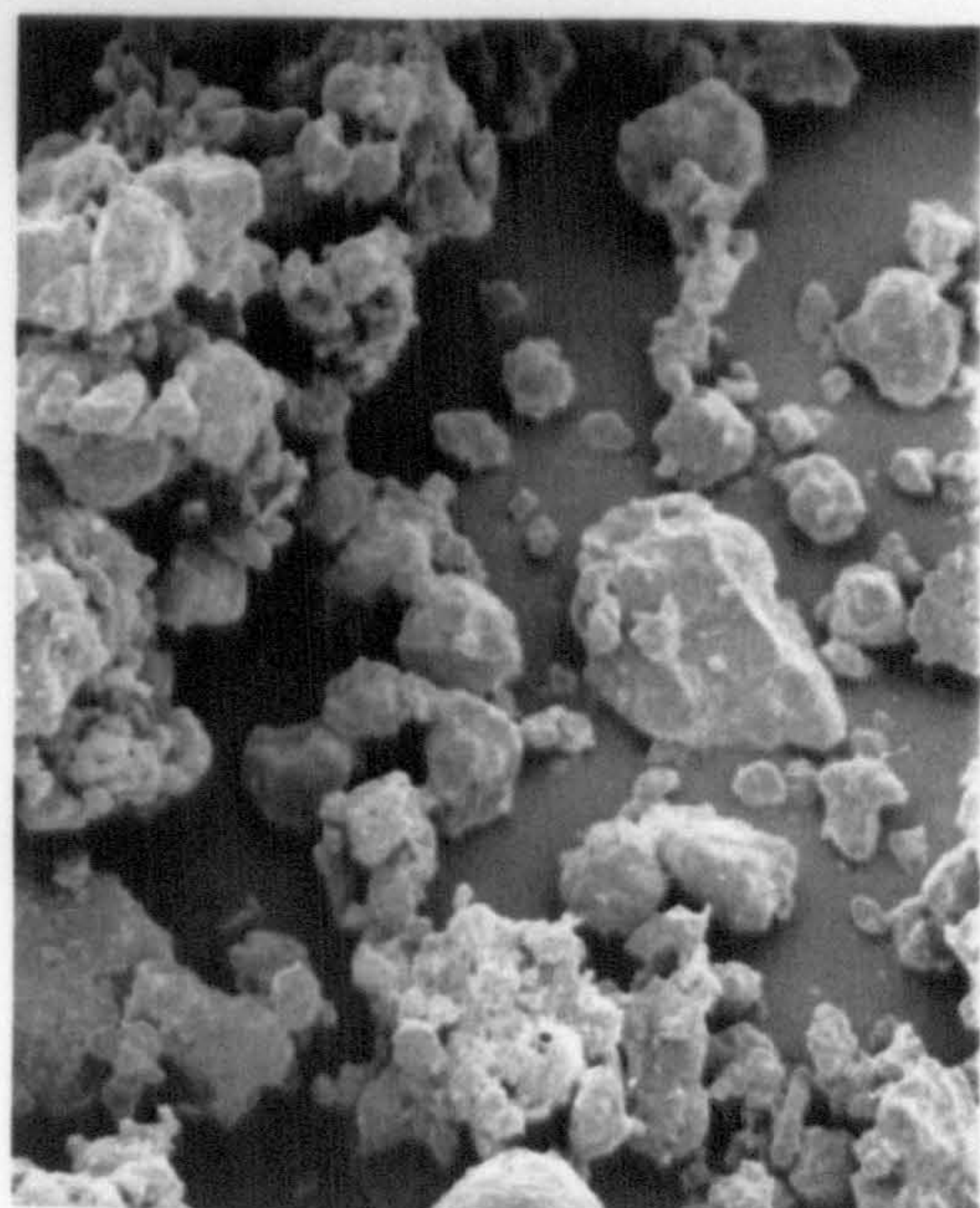
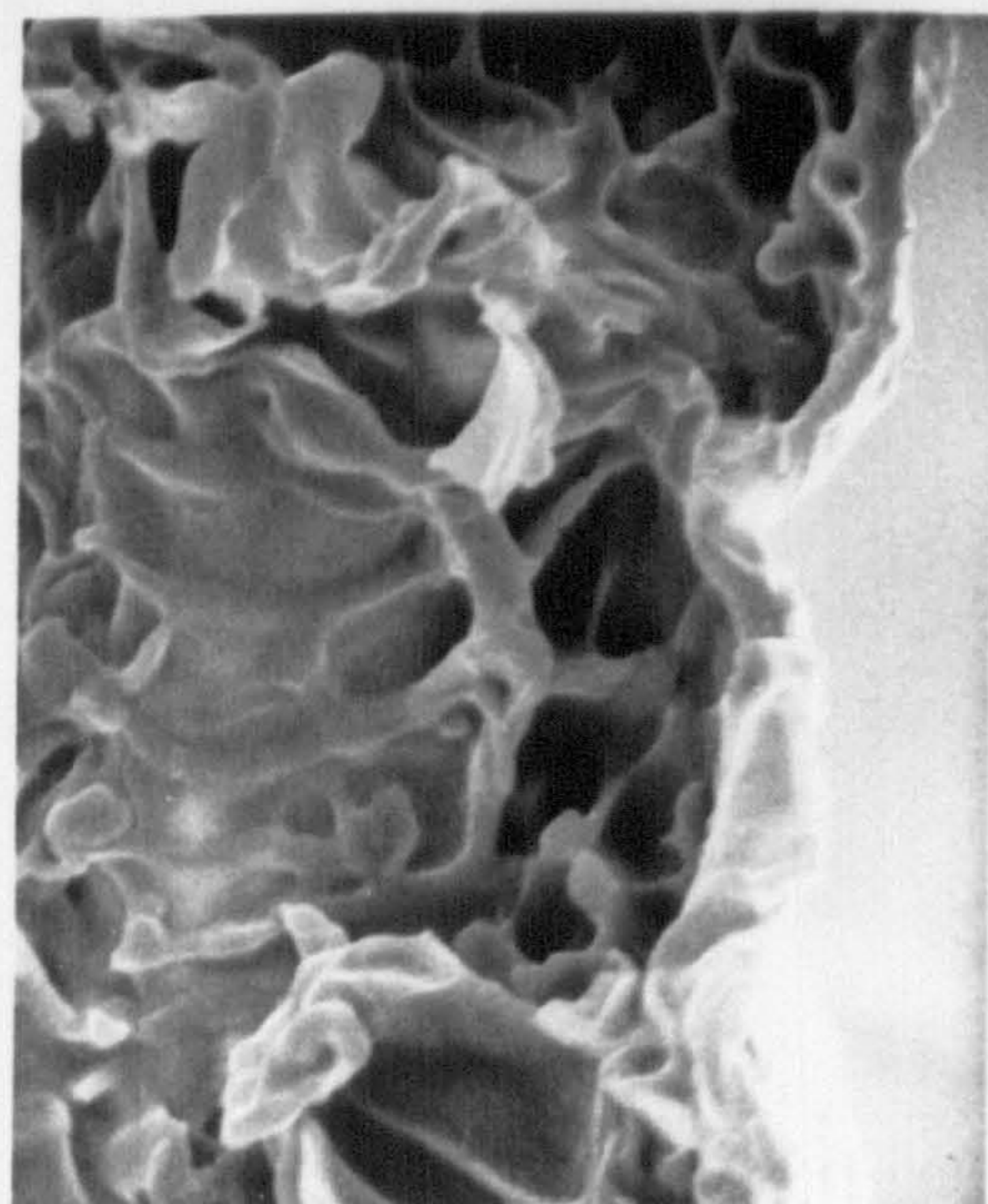
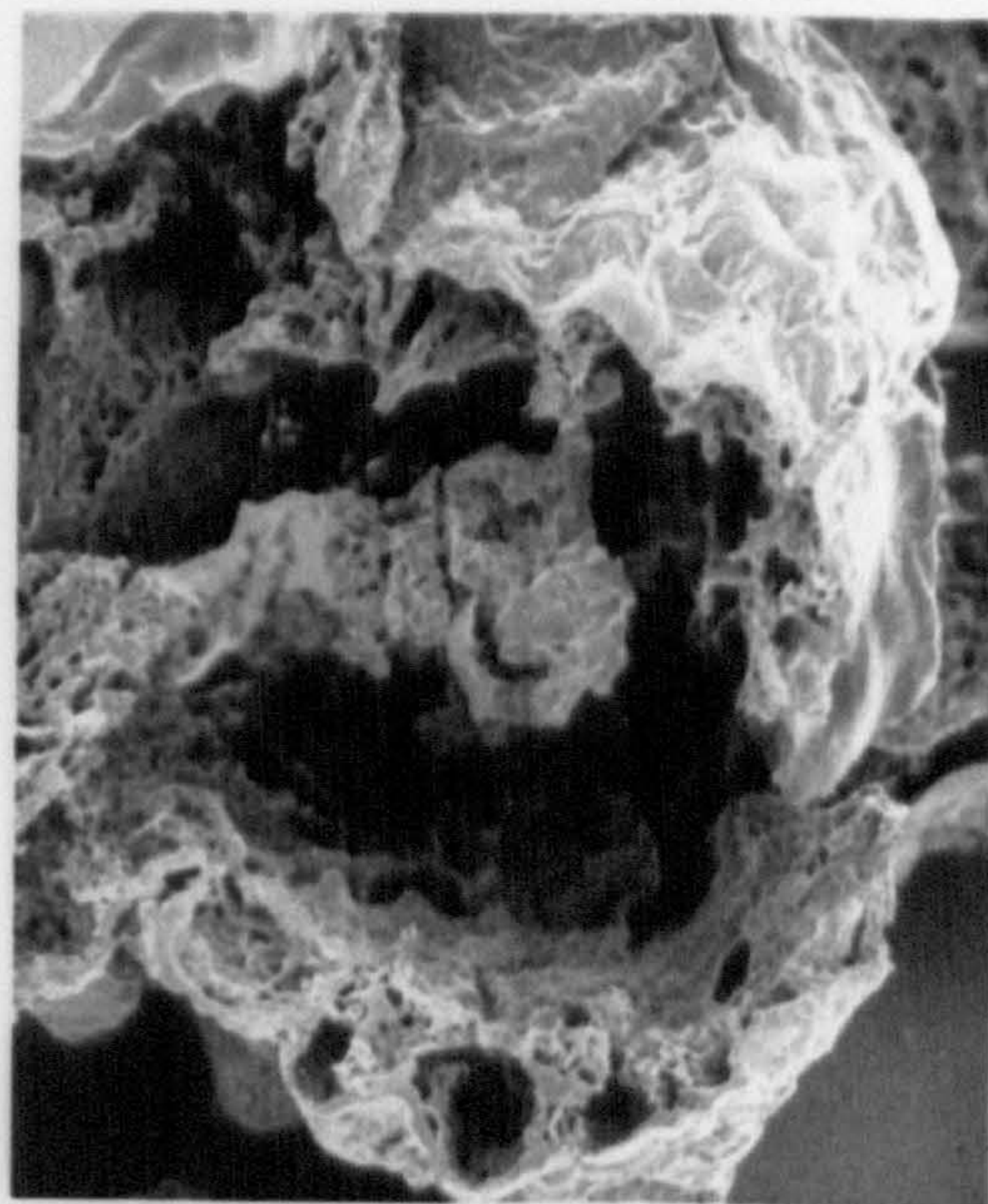
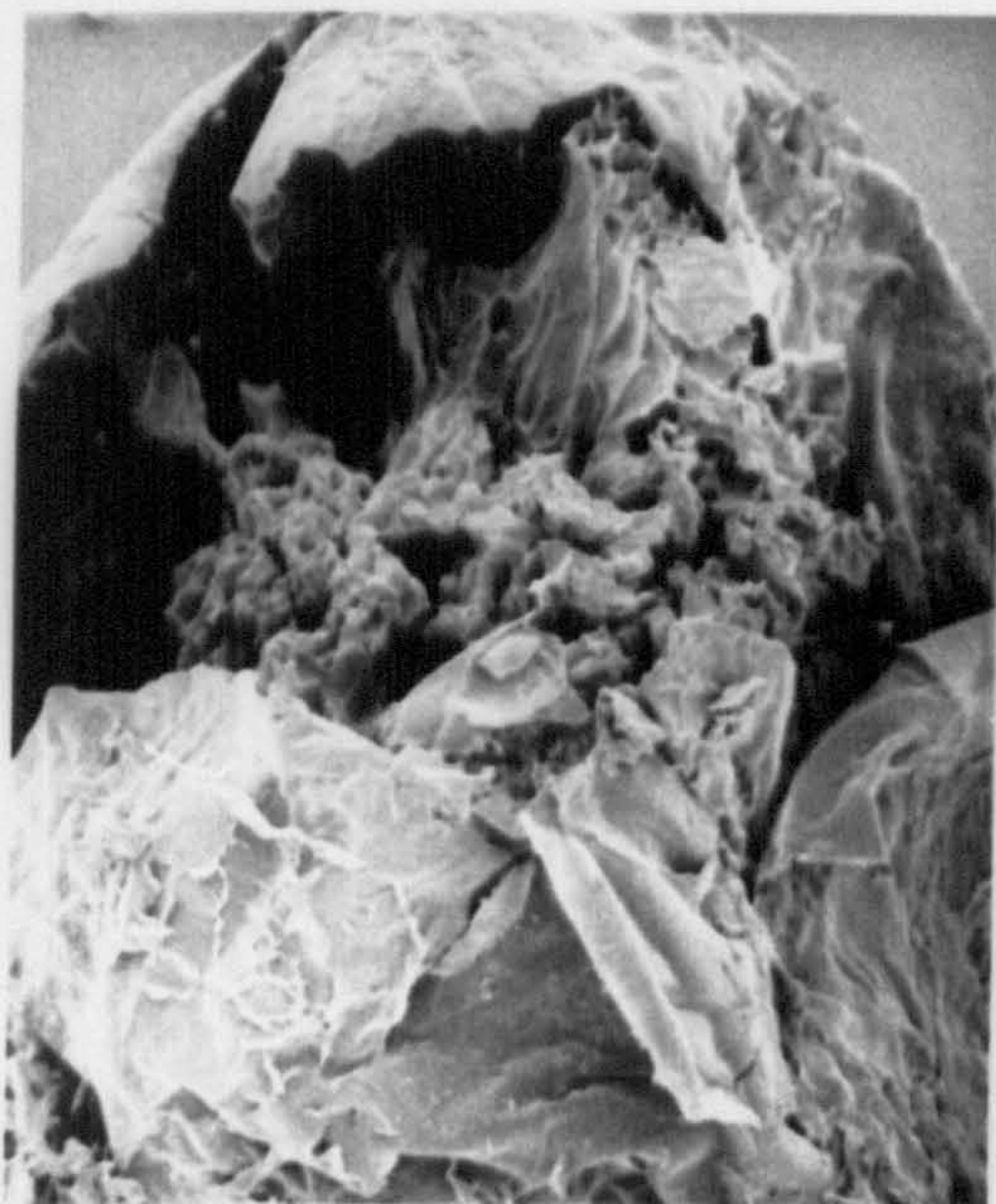
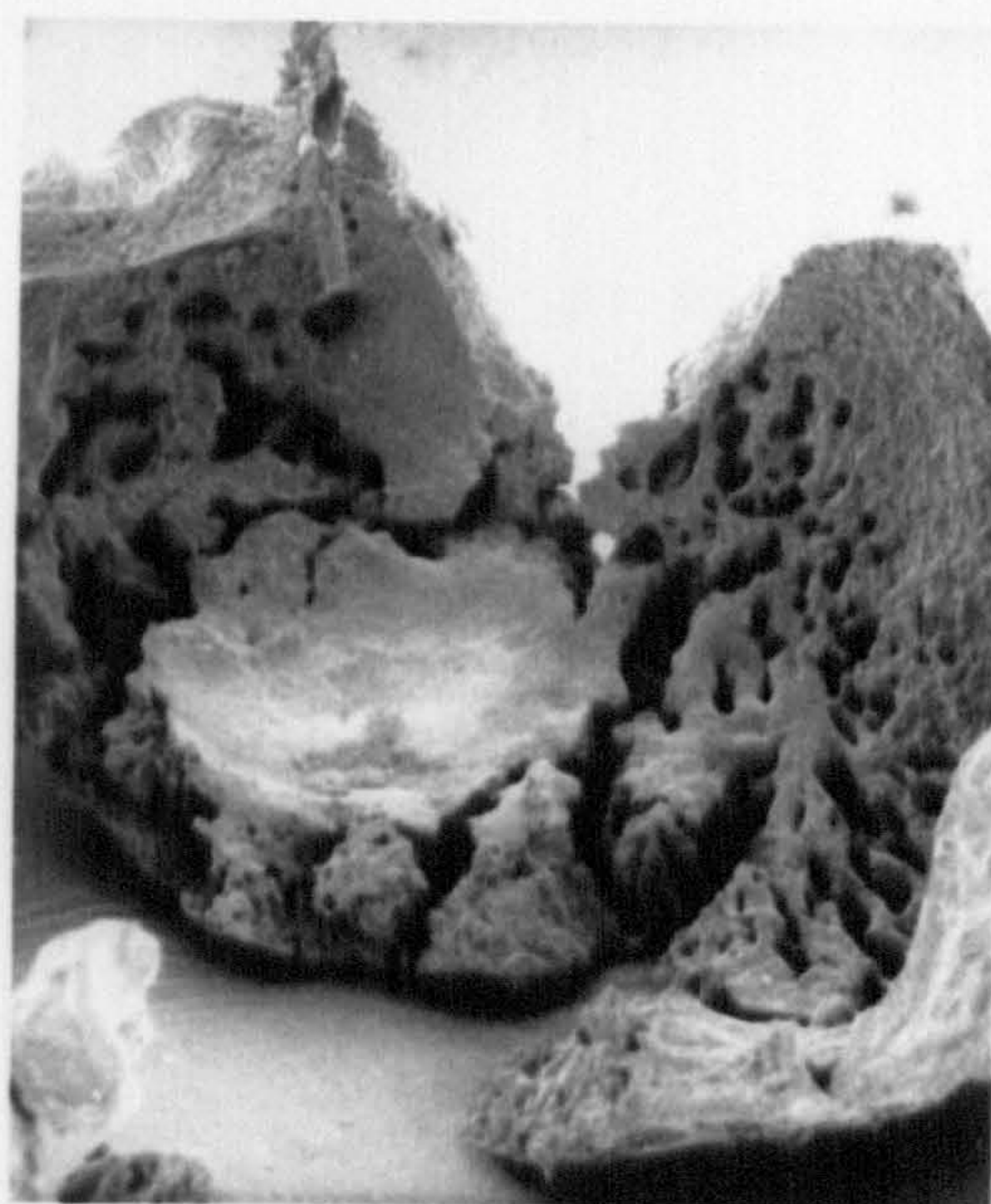
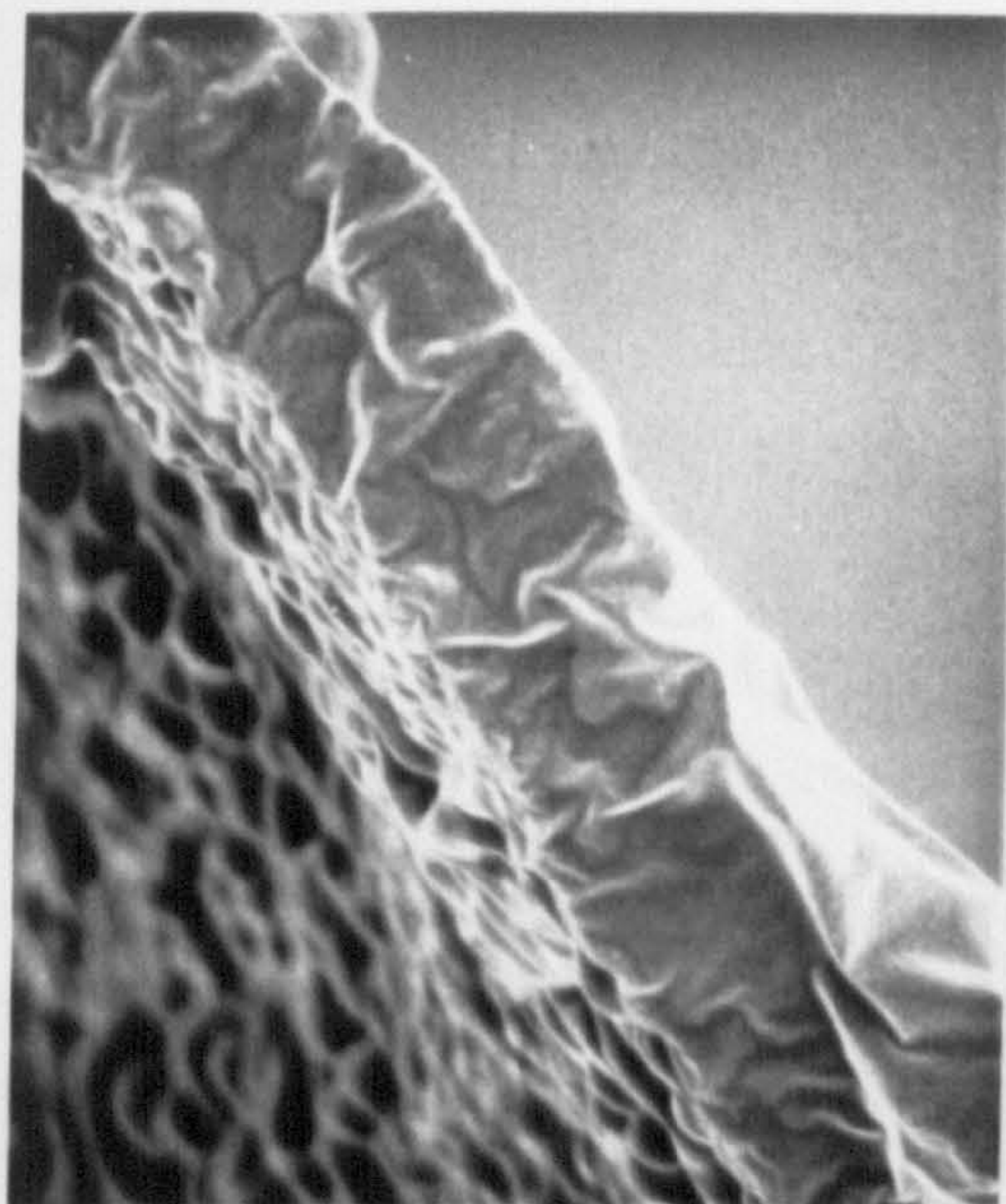
**B) Mag= x120**

**C) Mag= x250**

**D) Mag= x200**

**E) Mag= x1500**

**F) Mag= x500**



**Plate 4.**

**A) Mag= x400**

**B) Mag= x220**

**C) Mag= x400**

**D) Mag= x300**

**E) Mag x380**

**F) Mag= x1400**

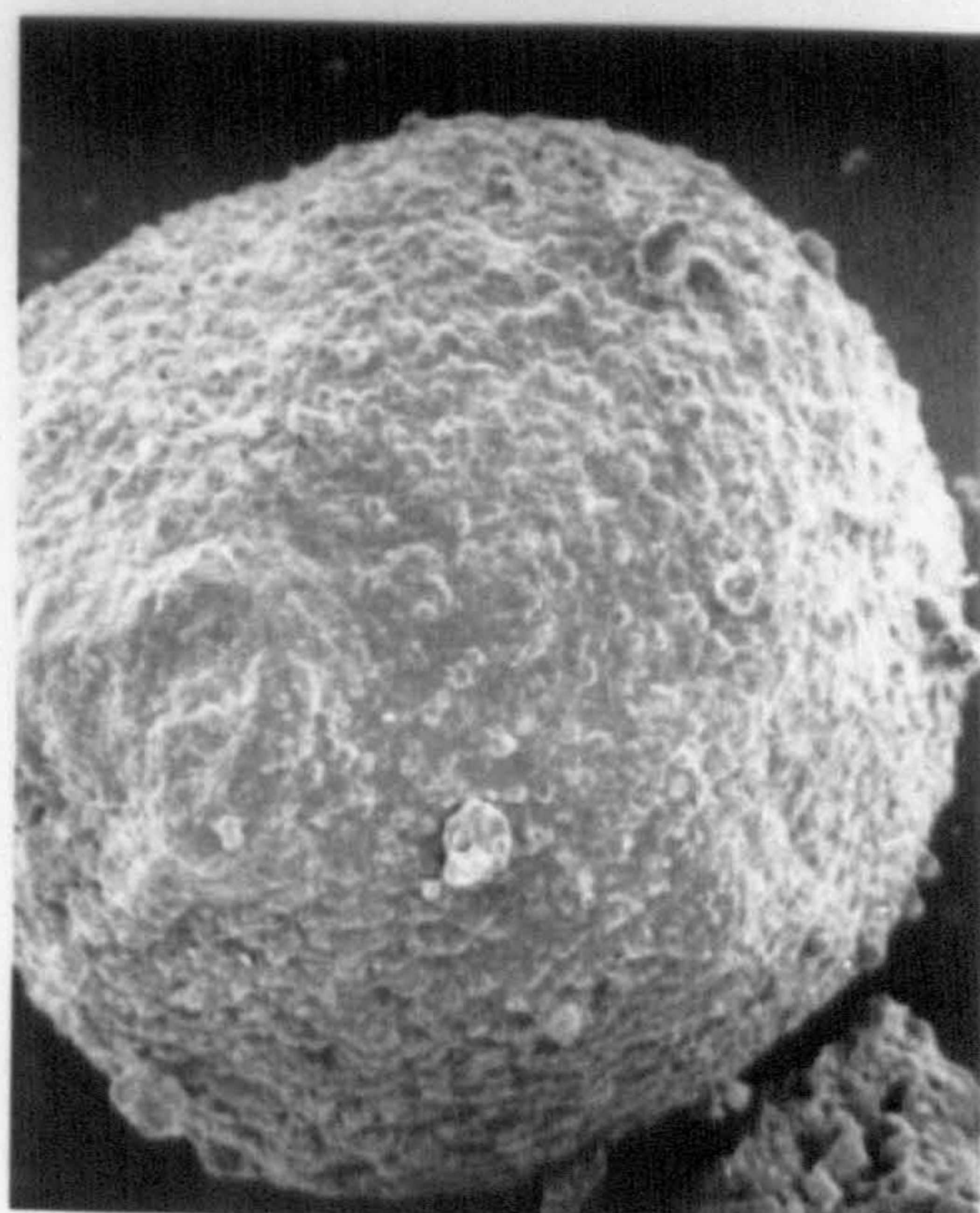
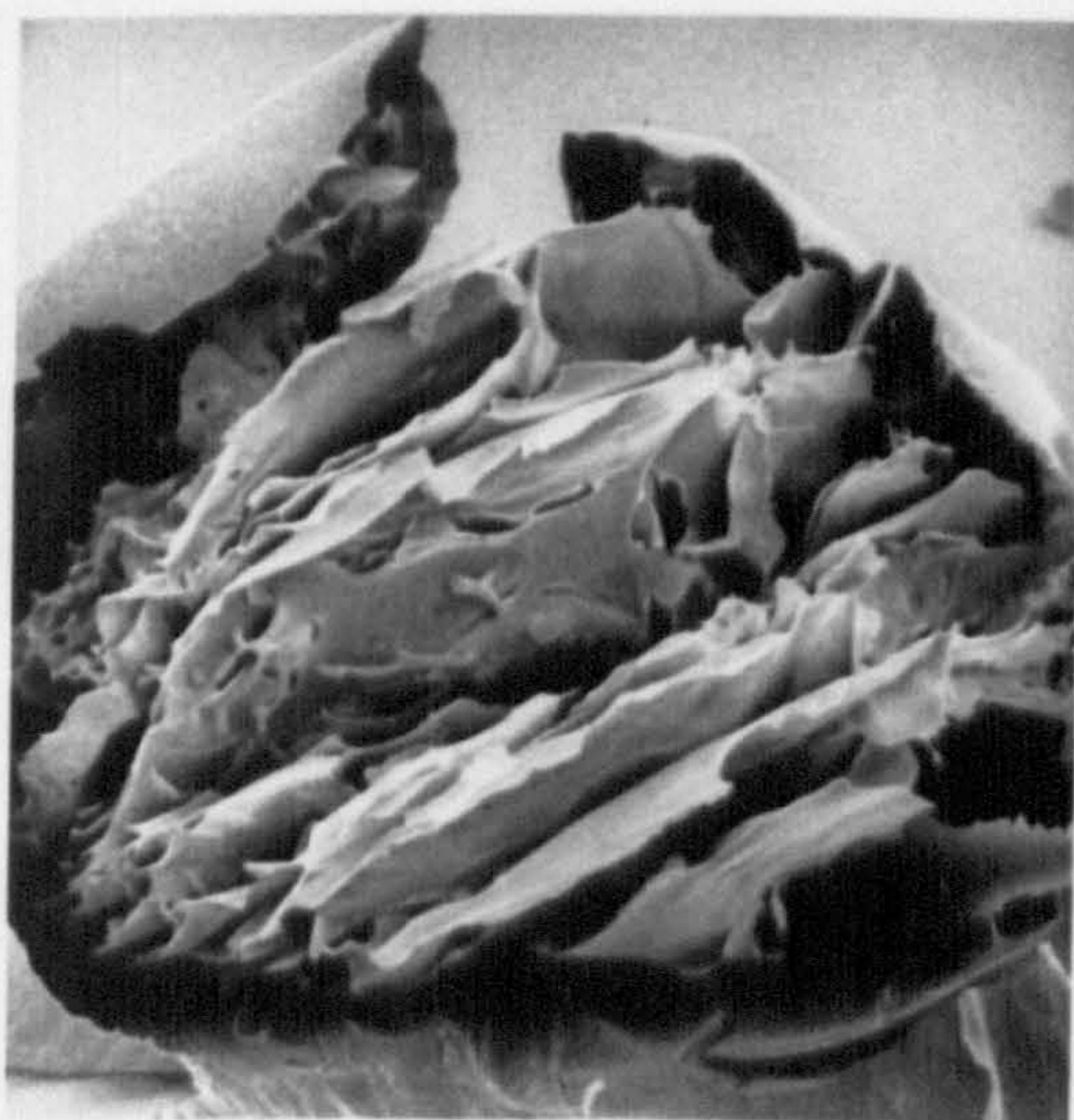
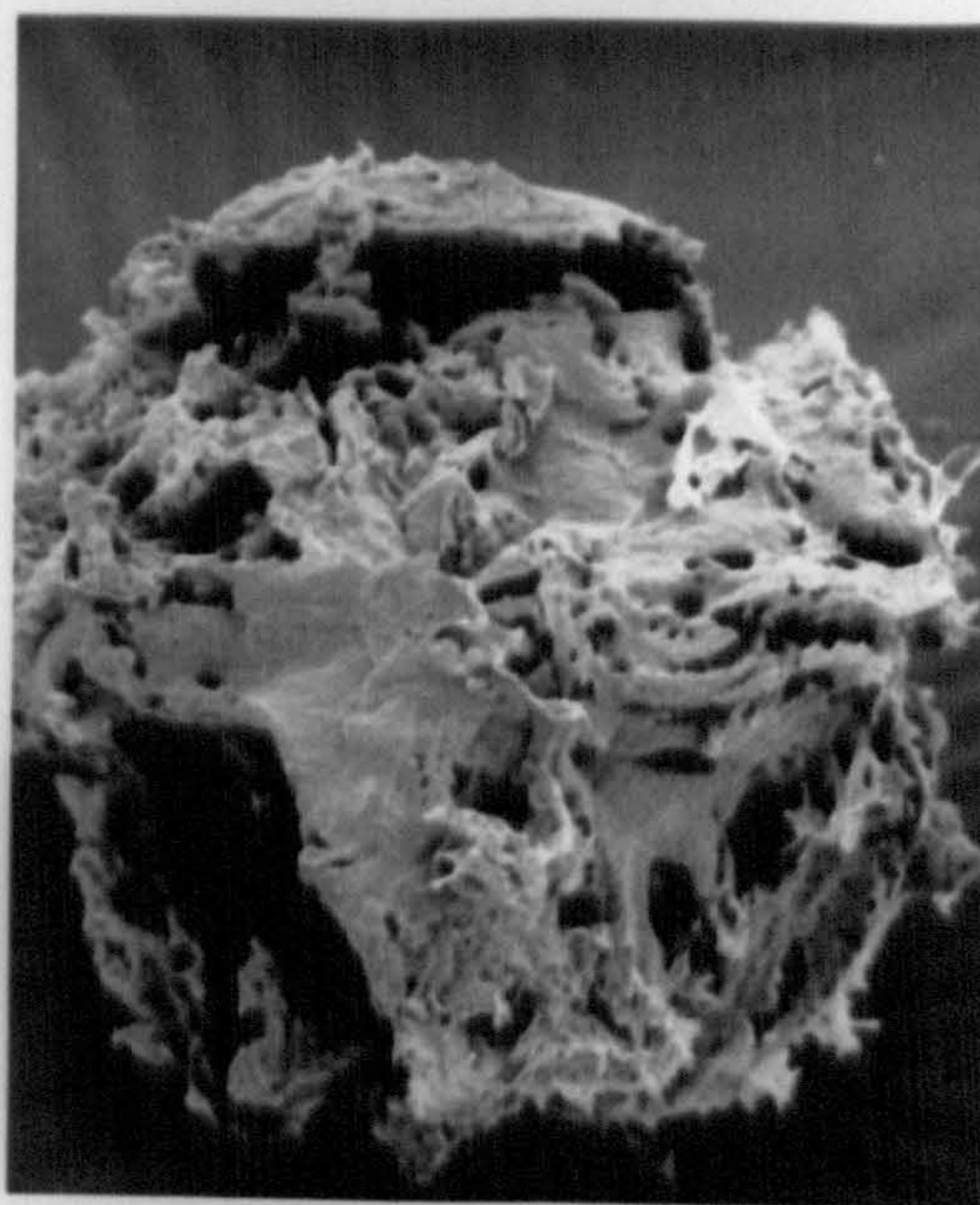
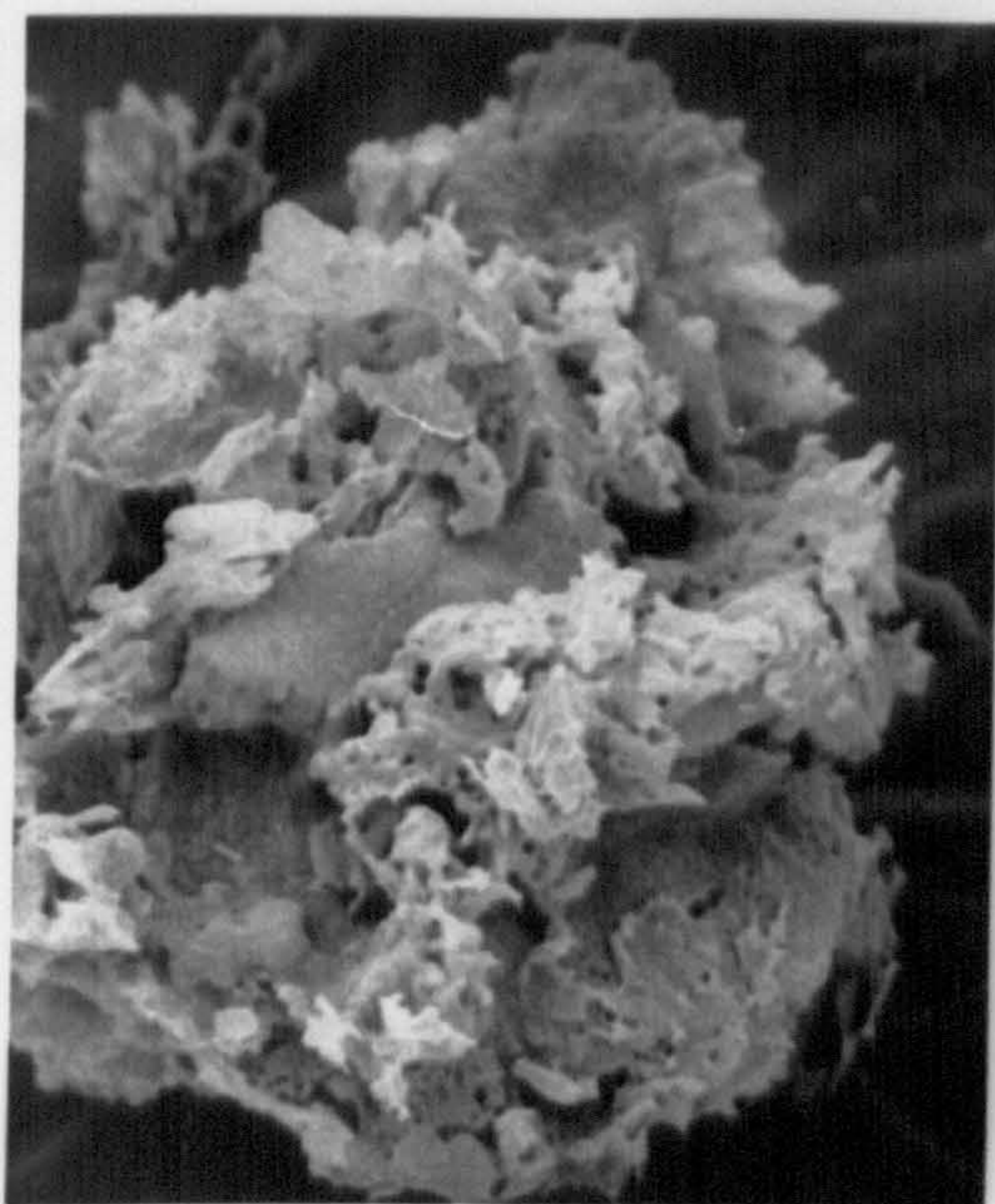
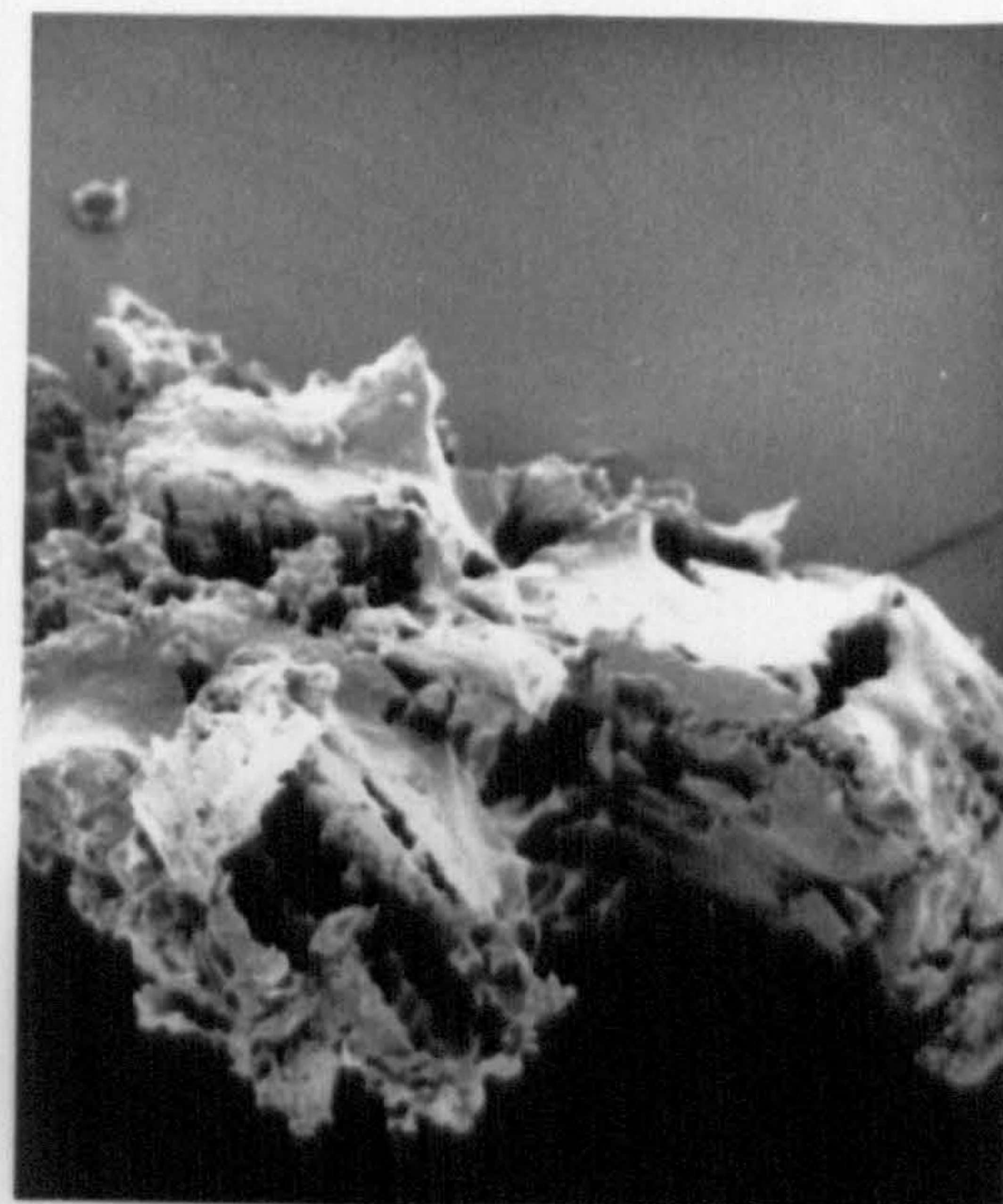
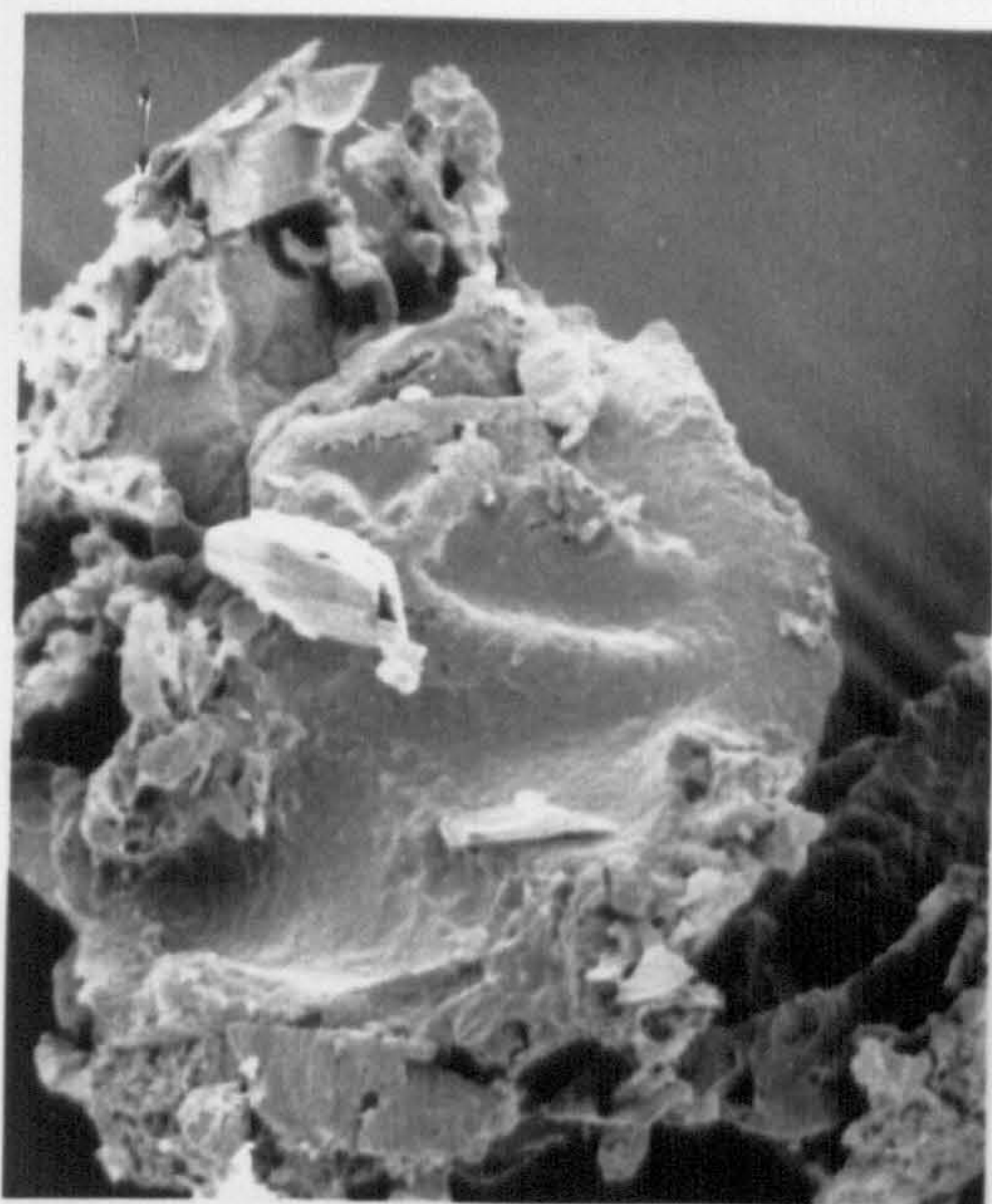


Plate 5.

A) Mag= x1300

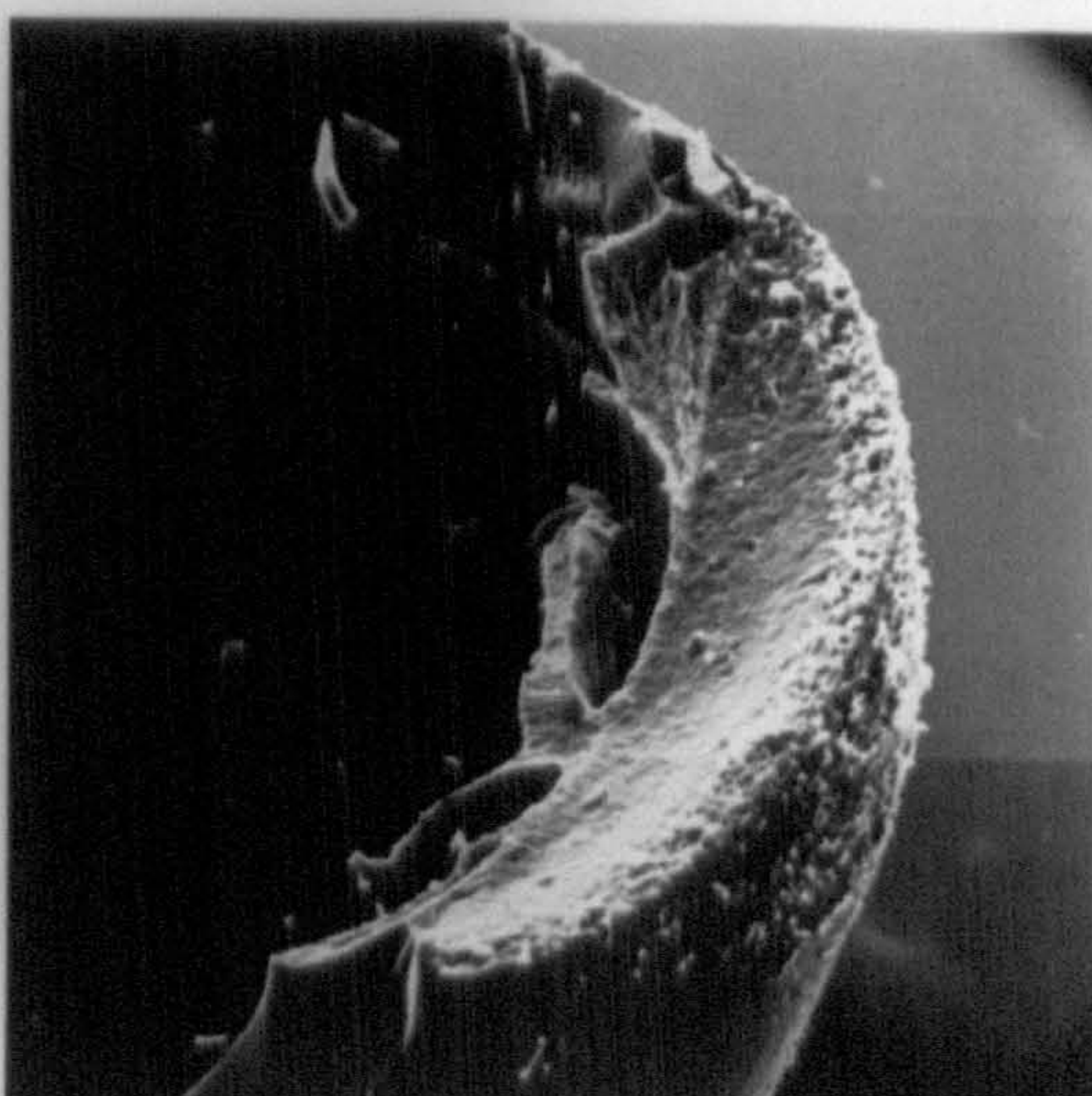
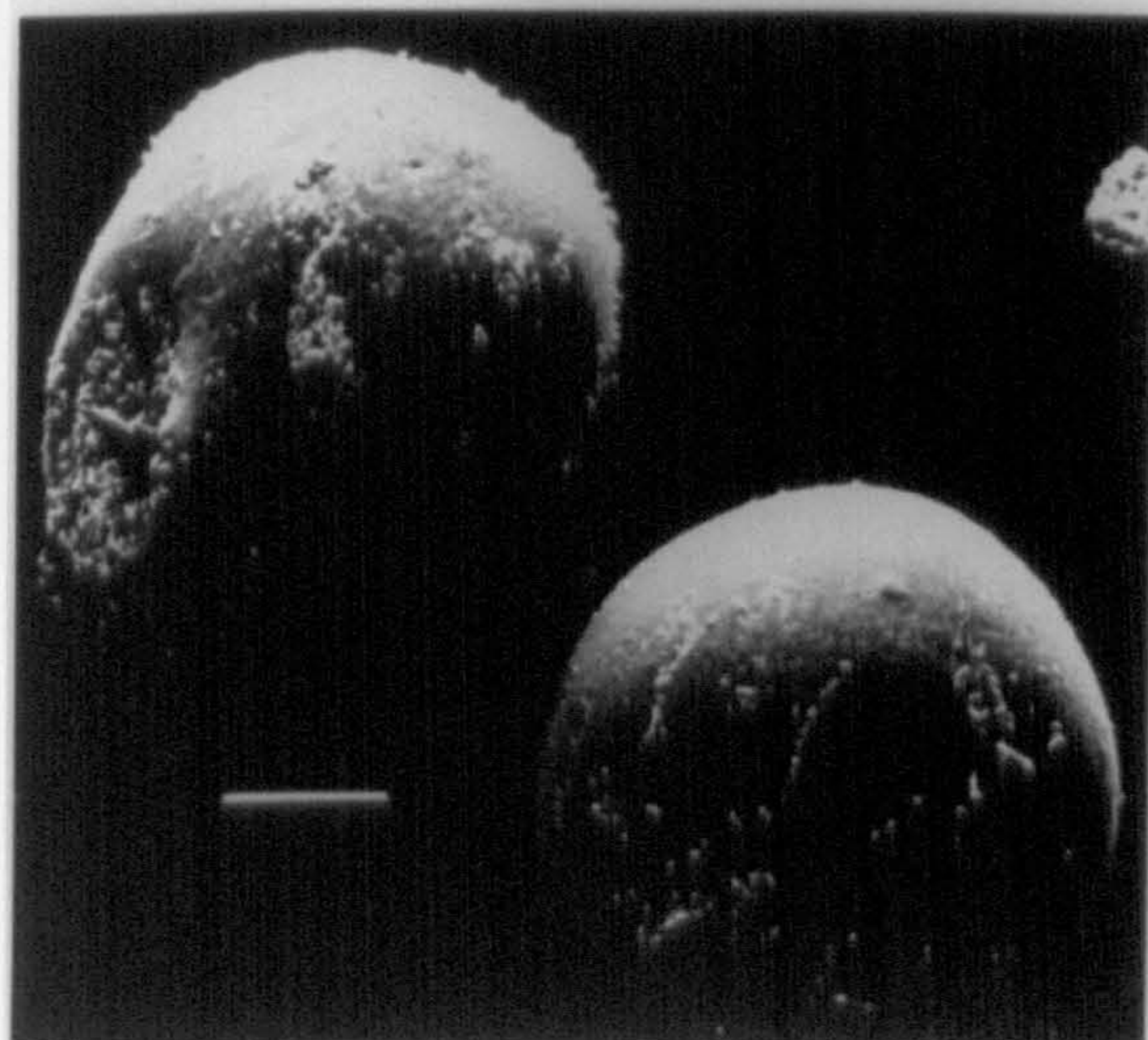
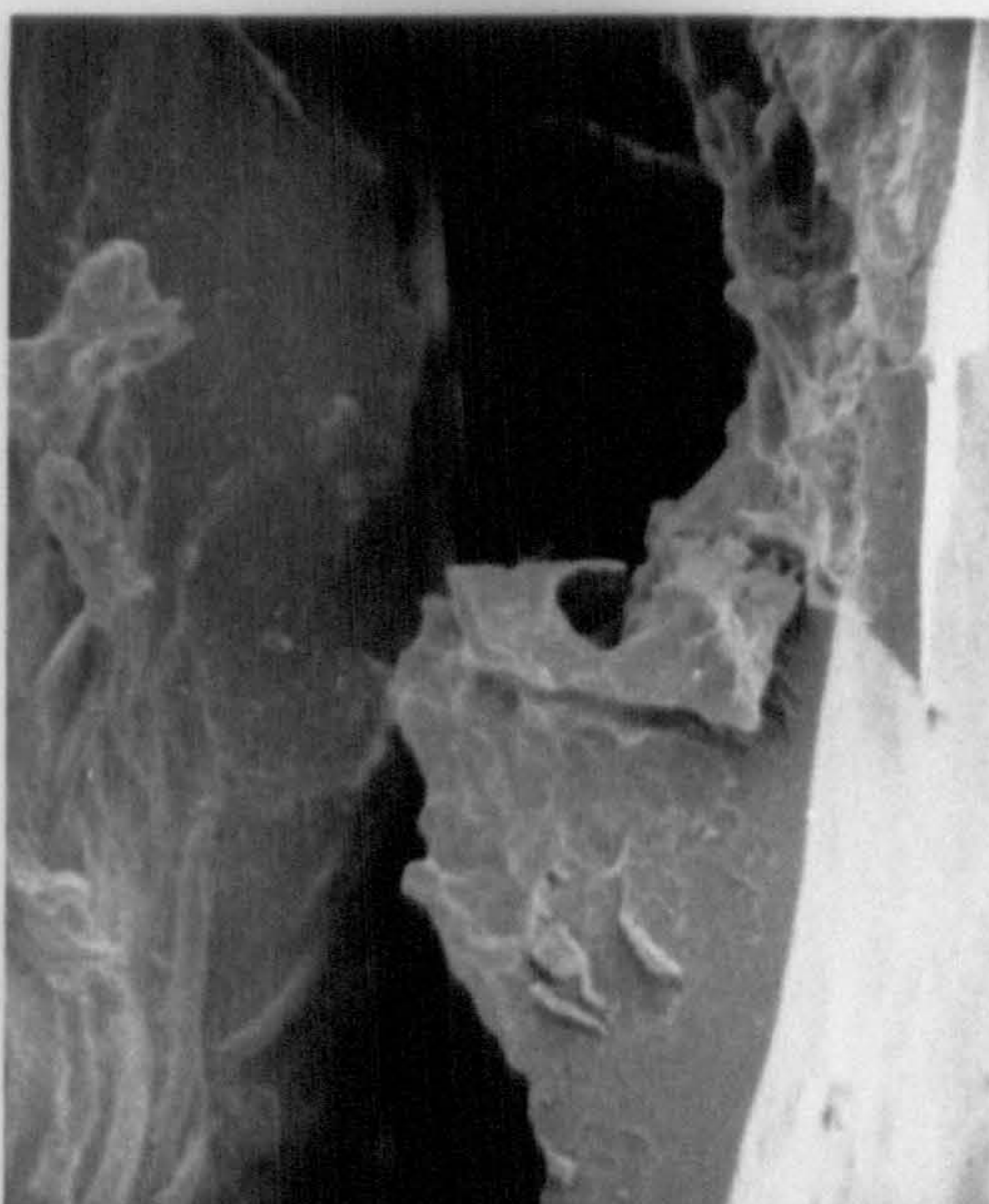
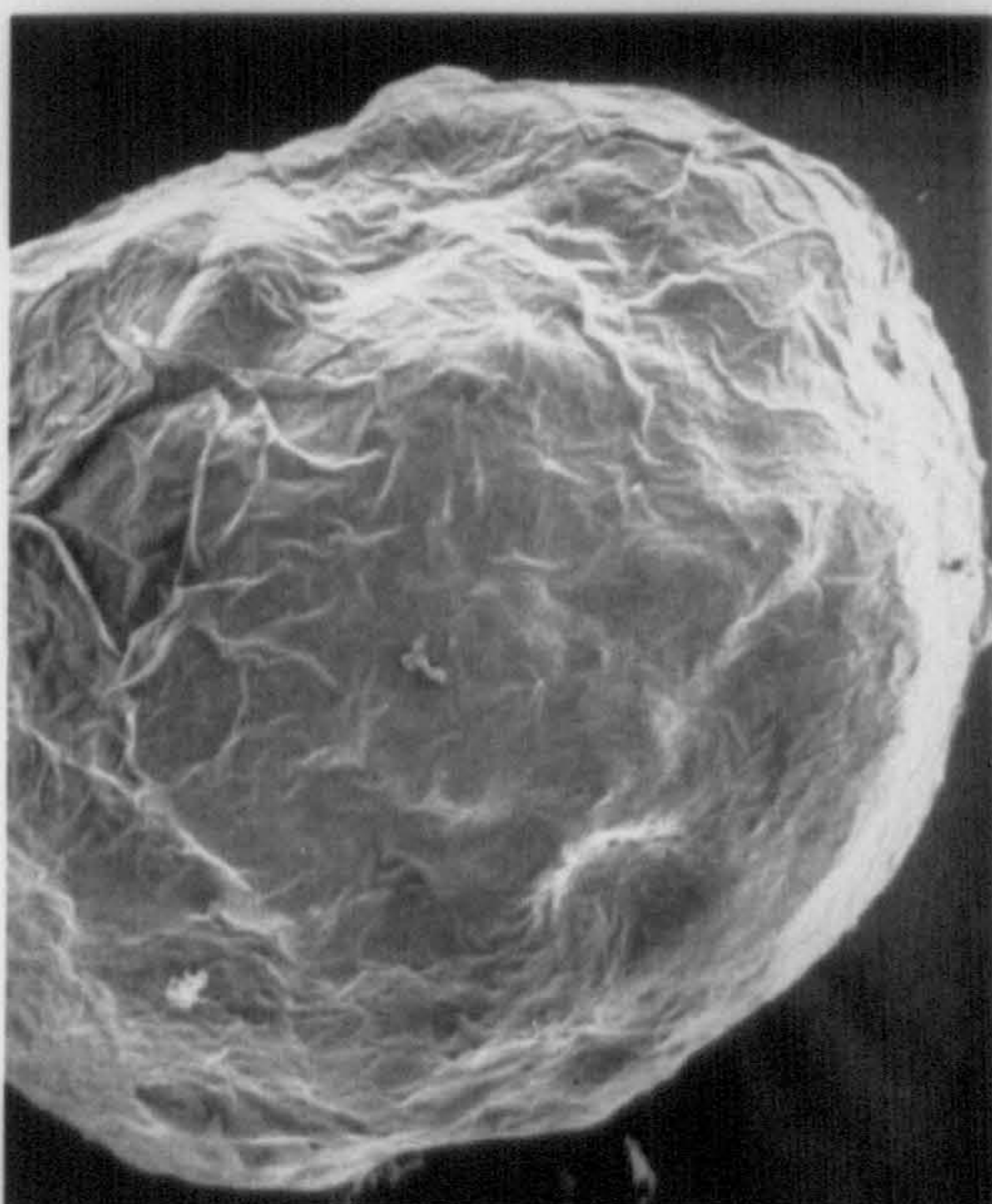
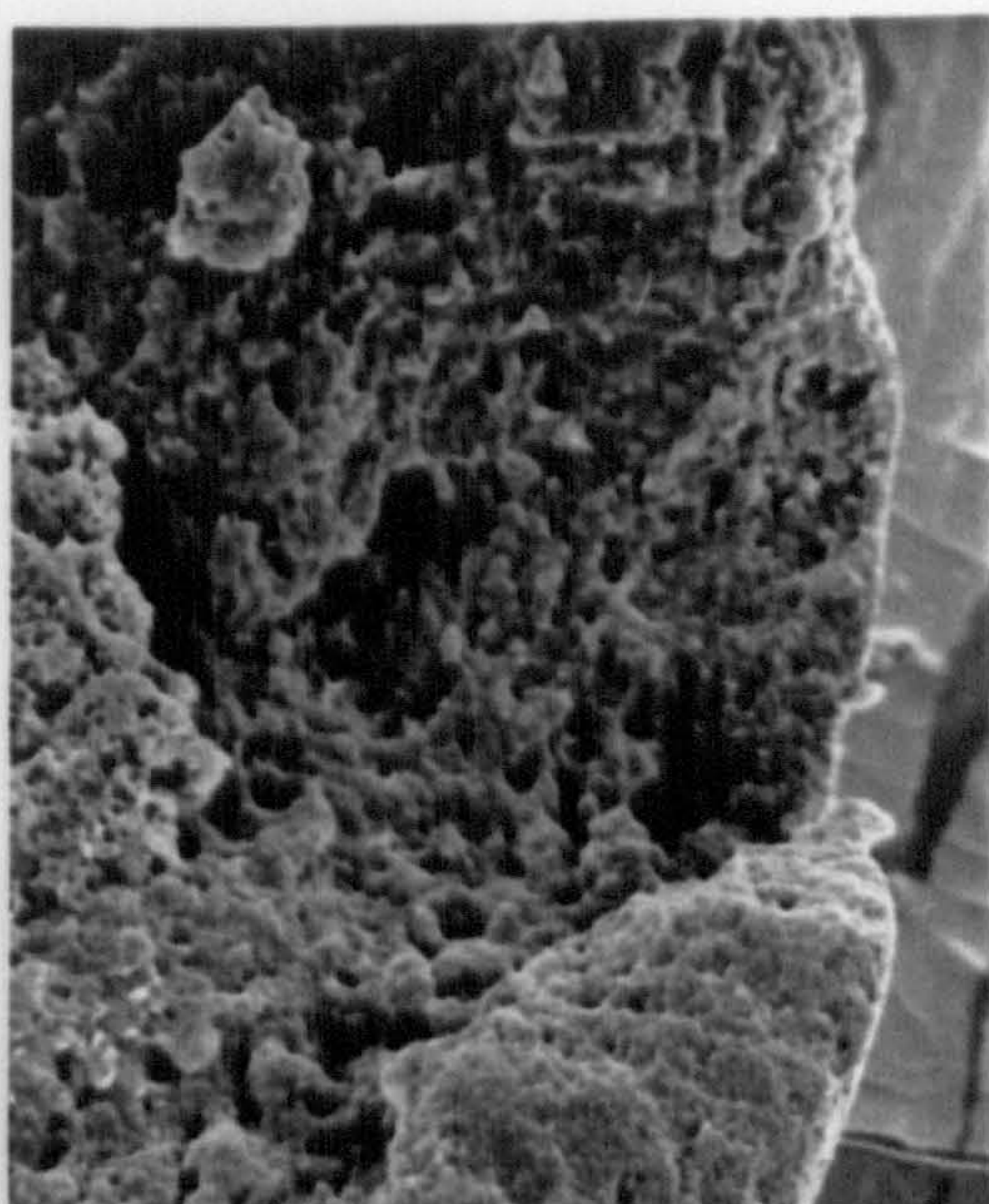
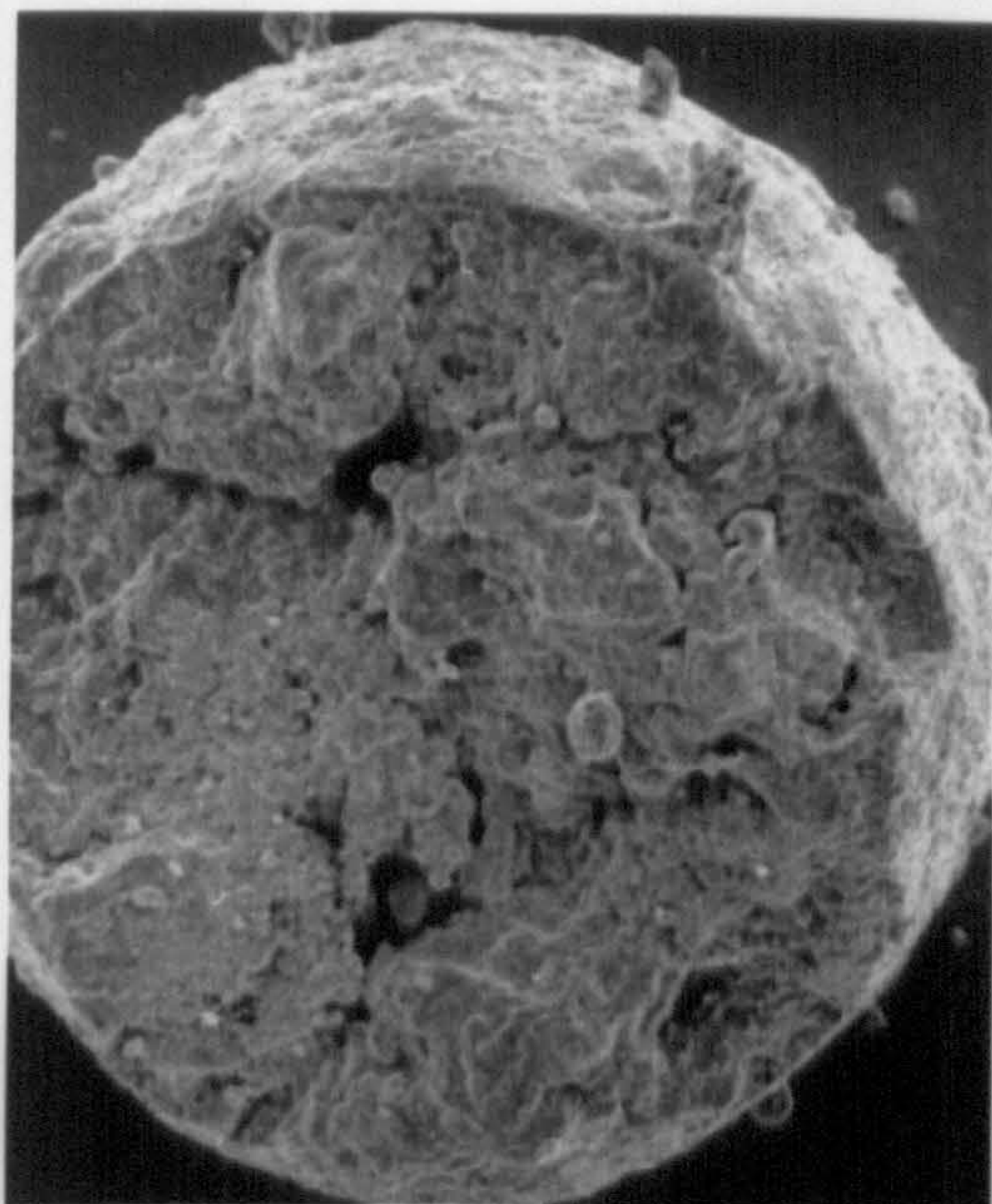
B) Mag= x2000

C) Mag= x1000

D) Mag= x1300

E) Mag= x450

F) Mag= x1000



**Plate 6.**

**A) Mag= x1300**

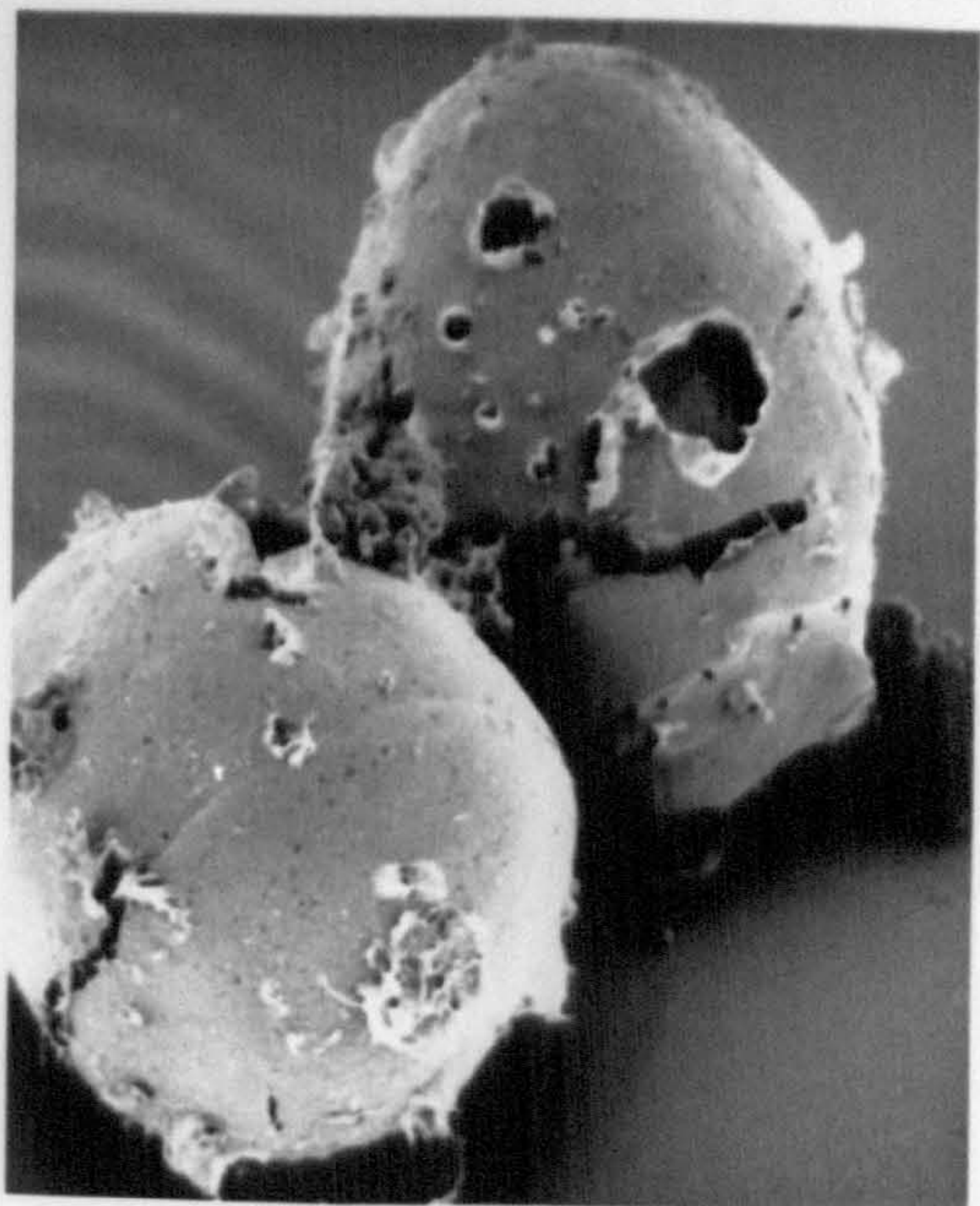
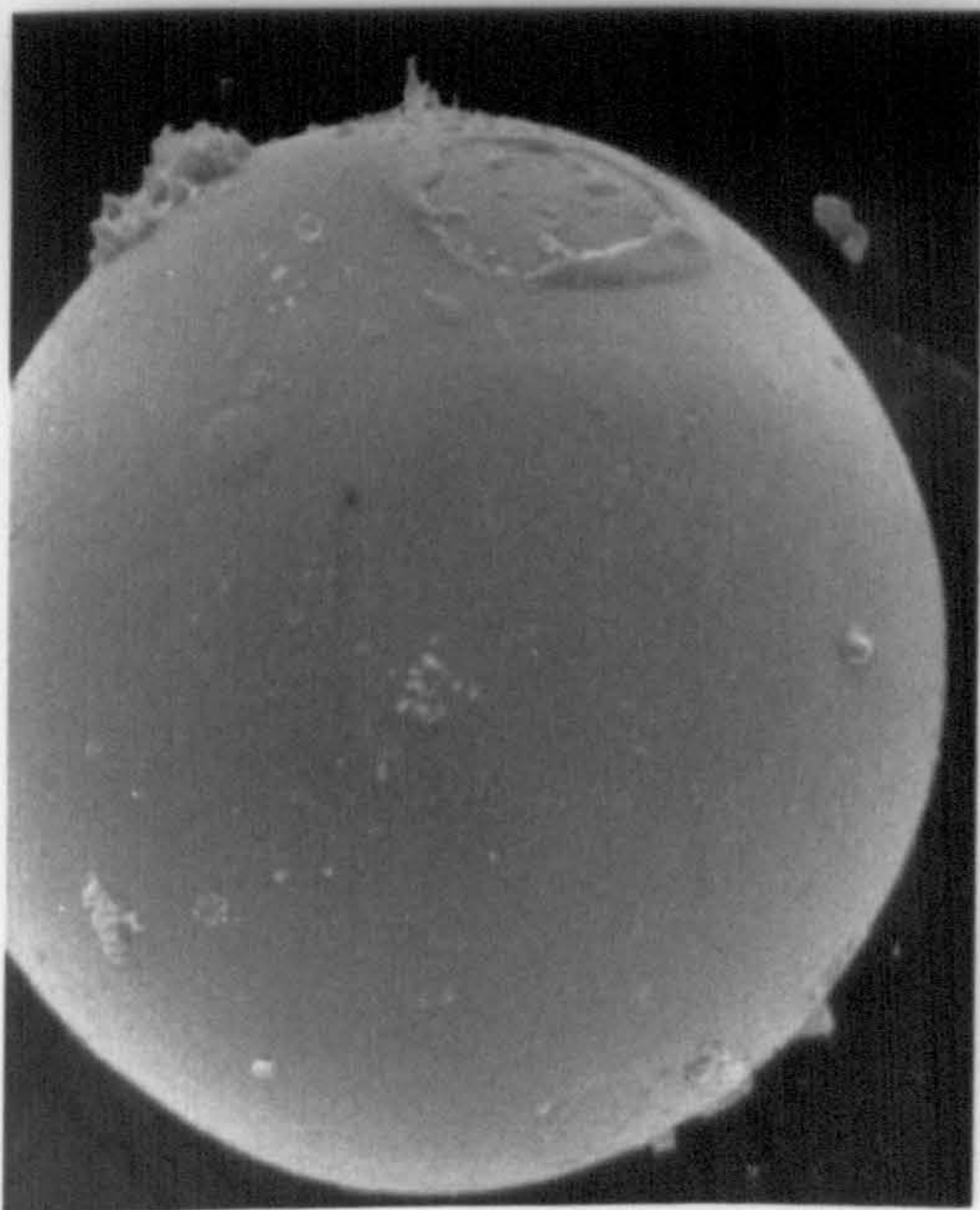
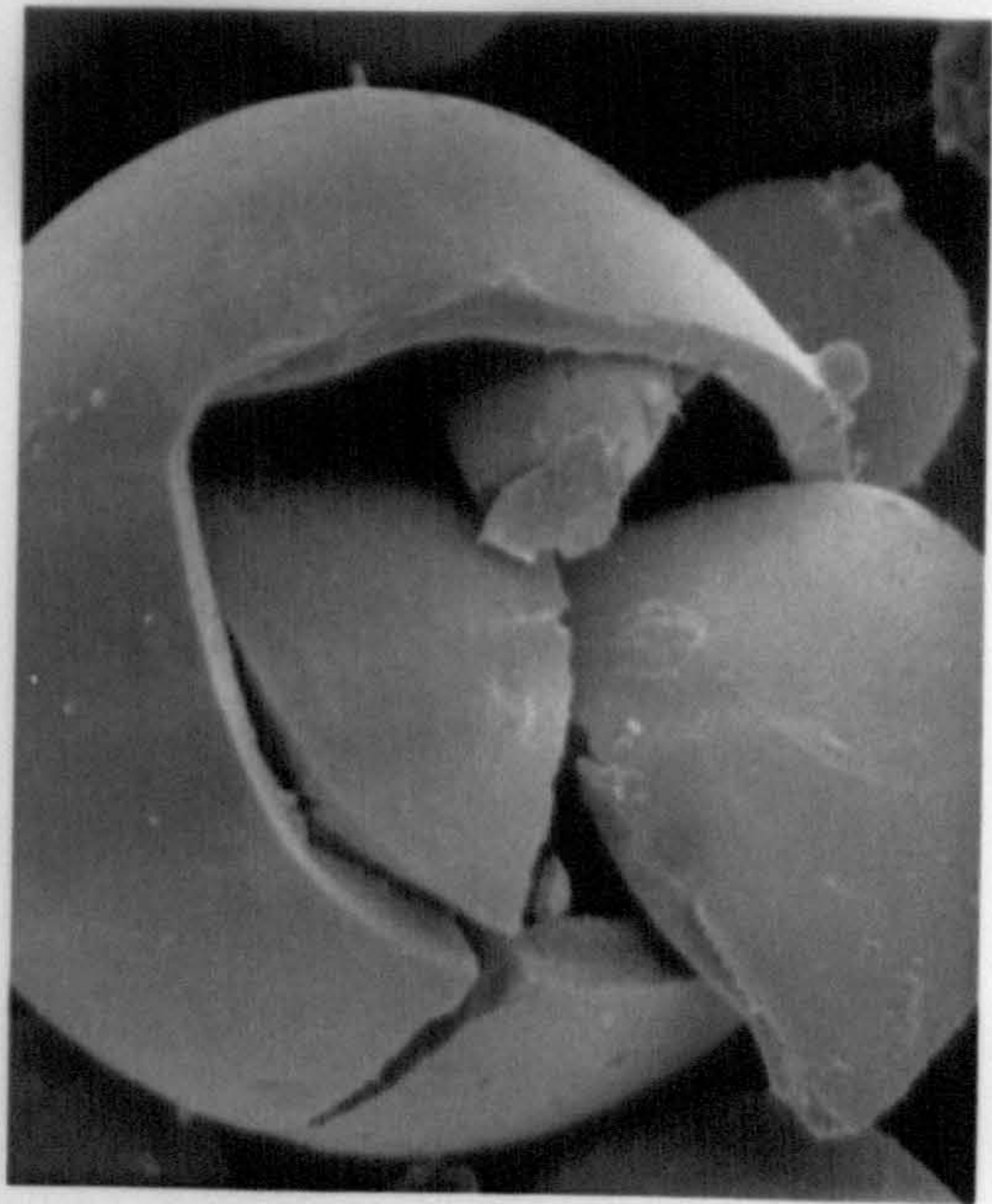
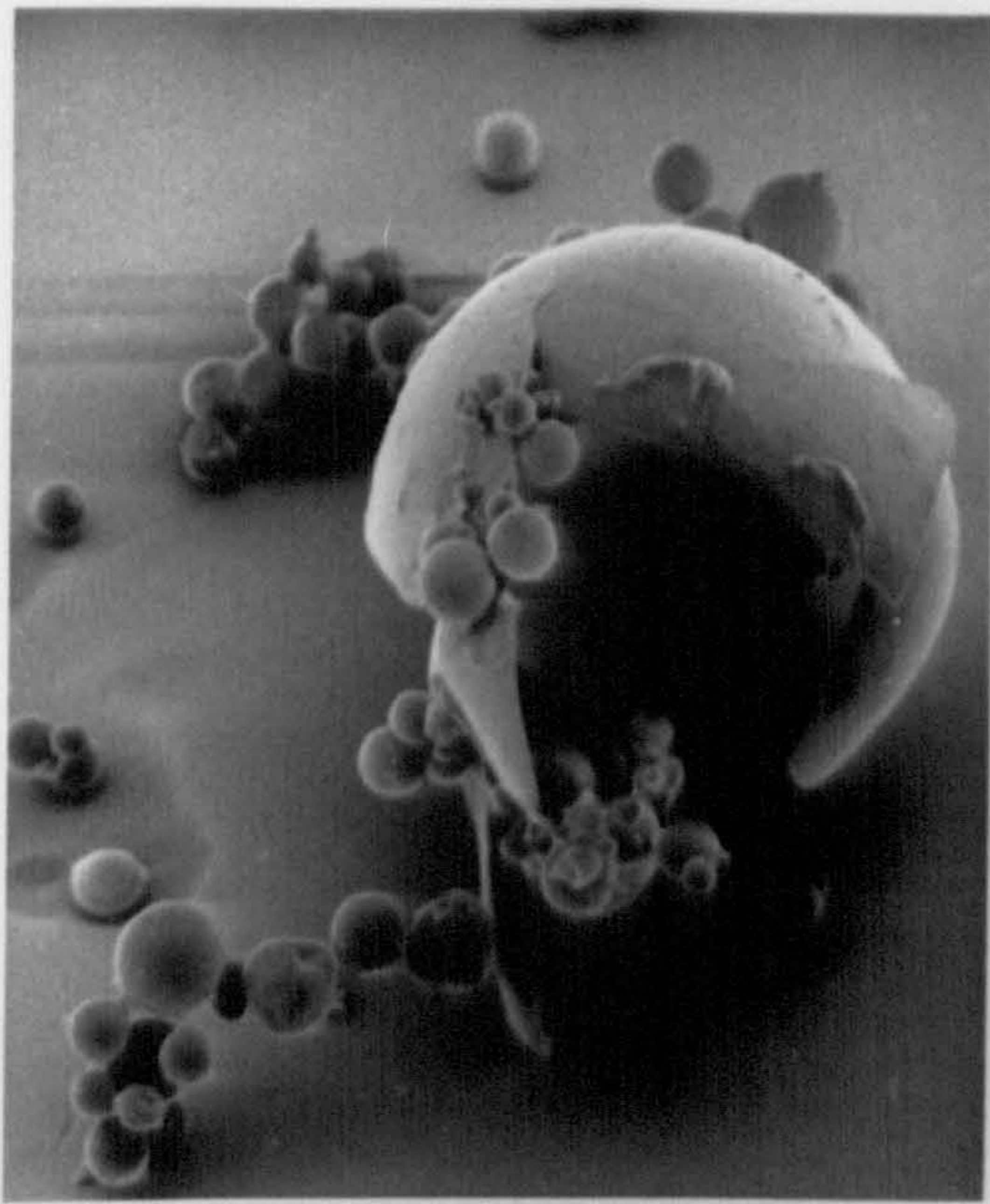
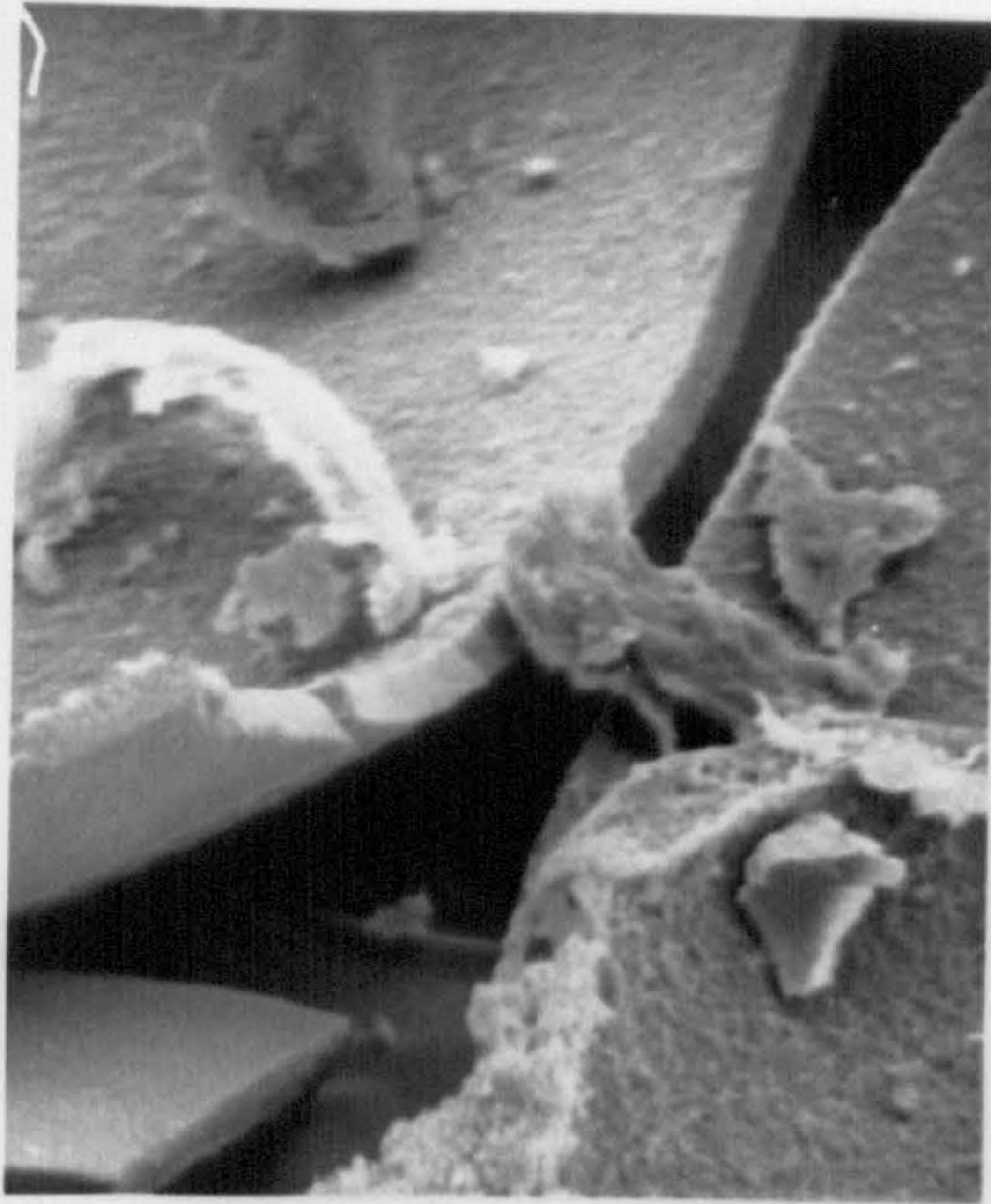
**B) Mag= x300**

**C) Mag= x450**

**D) Mag= x1000**

**E) Mag= x3000**

**F) Mag= x200**



**Plate 7.**

**A) Mag= x900**

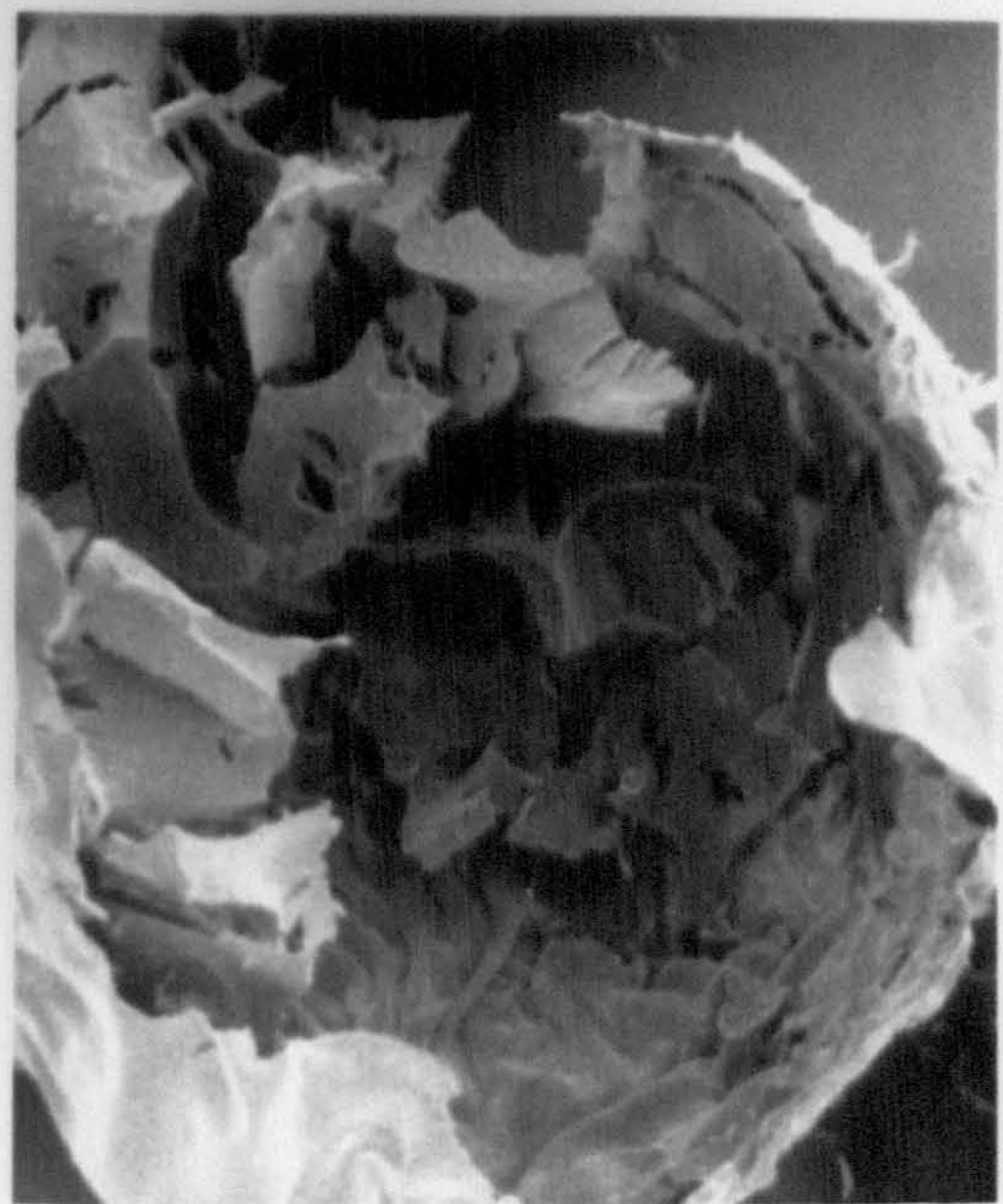
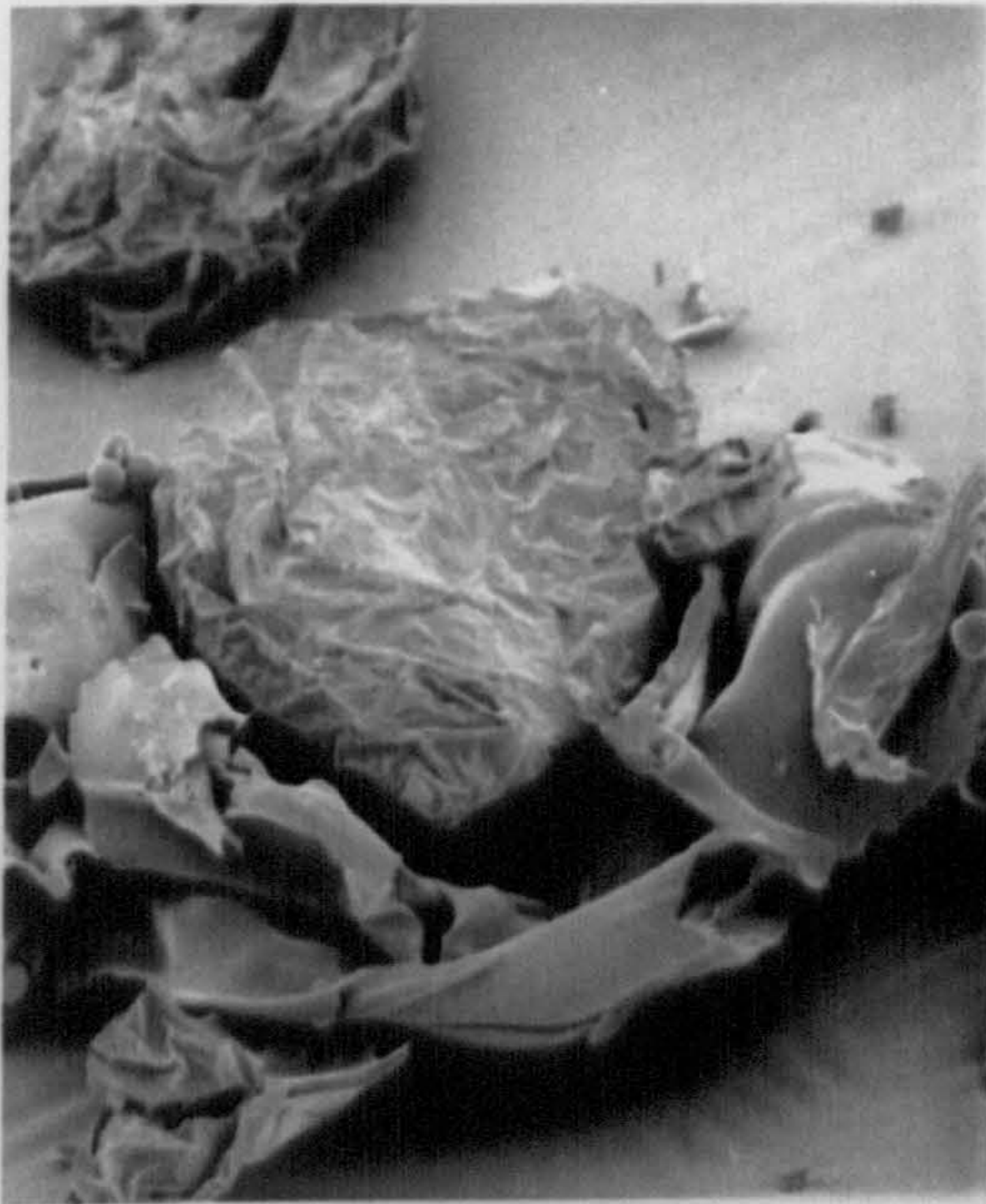
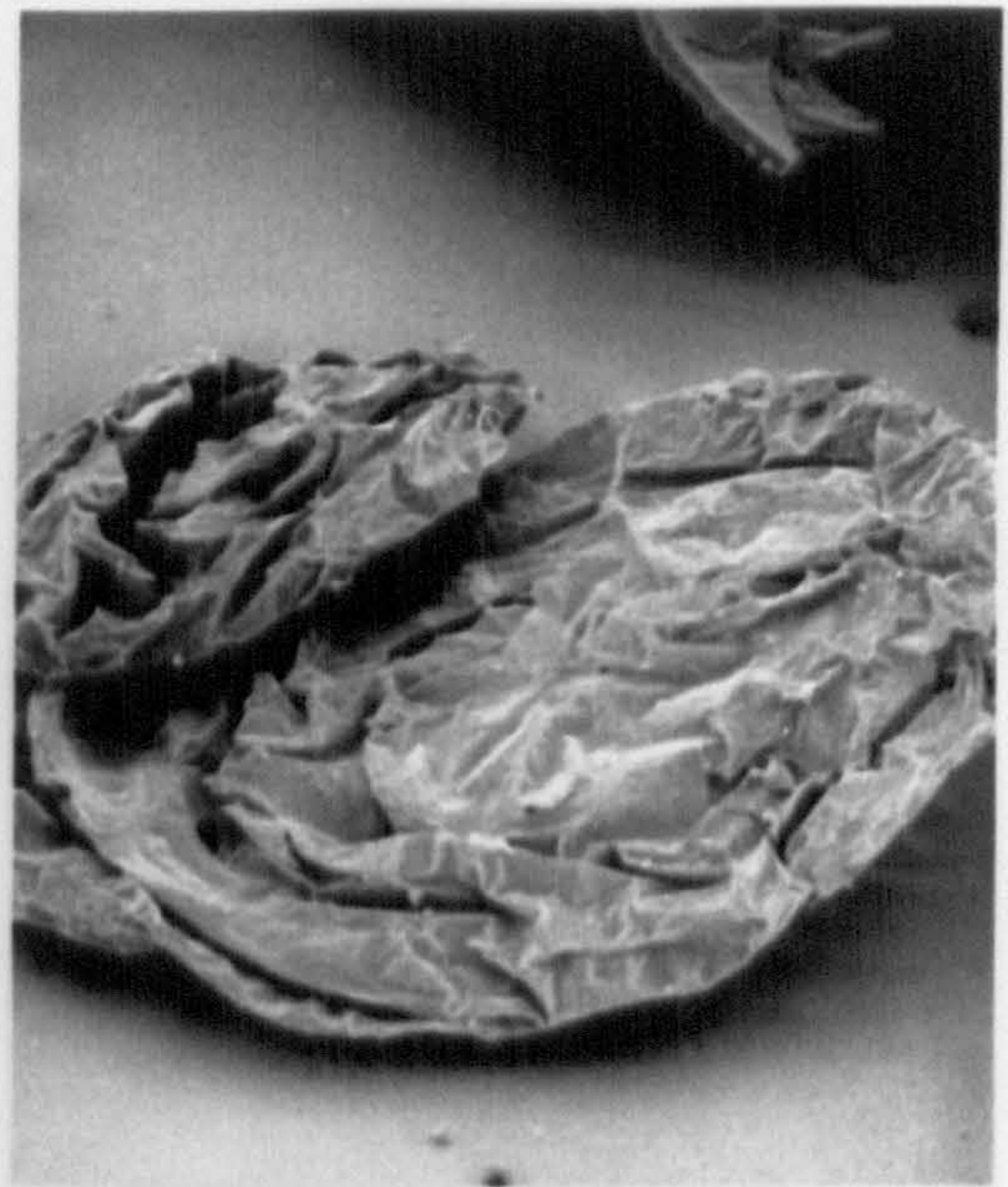
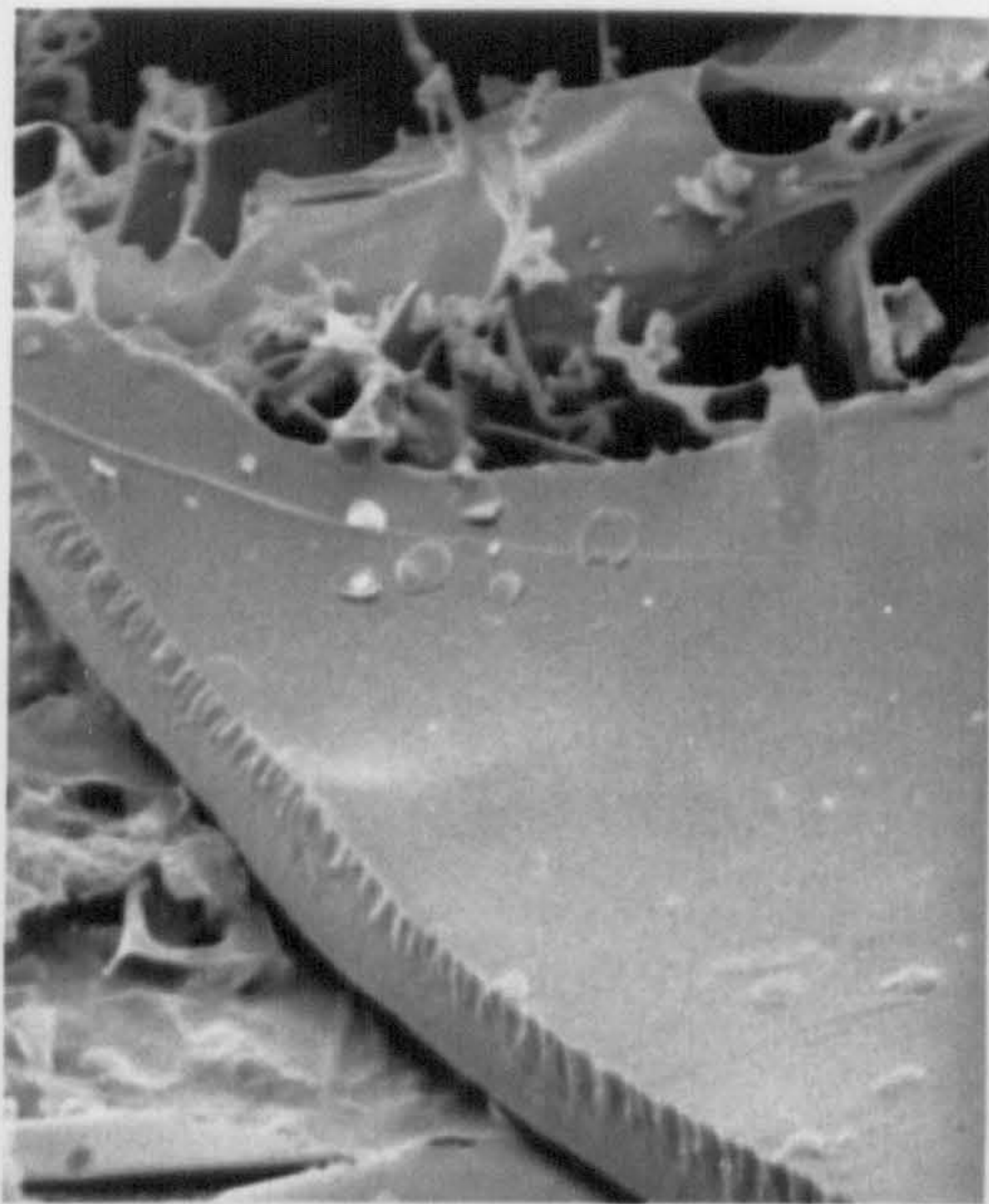
**B) Mag= x200**

**C) Mag= x200**

**D) Mag= x250**

**E) Mag= x200**

**F) Mag= x220**



An energy budget for the larvae of Penaeus monodon  
(Fabricius).

KURMALY, K., YULE, A.B. and JONES, D.A.

School of Ocean Science, University college of North Wales,  
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Third Party material excluded from digitalized copy.

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This material is also available in:  
*Aquaculture* 81.1 (1989): 13-25.

Title. Studies on effects of body size (5.64  $\mu$ g to 20 g dry weight) and temperature (15–35°C) on the metabolic rate of Penaeus monodon (Fabricius).

Authors: Kurmaly, K., Yule, A.B. and Jones, D.A.

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This material is also available in:  
*Marine Biology* 103.1 (1989): 25-30.

Comparative analysis of the growth and survival of Penaeus monodon (Fabricius) larvae, from protozoa 1 to postlarva 1, on live feeds, artificial diets and on combinations of both.

Kurmaly, K., Jones, D.A., Yule, A.B. and East, J.

School of Ocean Science, University college of North Wales,  
Menai Bridge, Gwynedd, U.K. LL59 5EY.

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This material is also available in:  
*Aquaculture* 81.1 (1989): 27-45.

Gut evacuation time, assimilation efficiency and energy budgets for juvenile Penaeus monodon. (Penaeidae: Decapoda).

KURMALY, K., JONES, D.A., CANAVATE, J.P., YULE, A.B. AND GROVE, D.

School of Ocean Science, University college of North Wales,  
Menai Bridge, Gwynedd, U.K. LL59 5EY.

## Abstract

The time (h) for 50% of faeces to be evacuated from the gut (GET) of juvenile Penaeus monodon was affected by diet type and increased with percentage body weight ingested (%BWI), and wet weight of prawn. The percentage of body weight ingested each day was a reciprocal function of body weight given by:

$$\%BWI = 1/(0.0254 + 0.0045 \times WW)$$

Irrespective of the amount defaecated with time, assimilation efficiency (AE2%), estimated by the 'ratio' method (Conover, 1966a,b), ranged between 60-80% and was independent of %BWI, wet weight of prawn and seawater preparations of cholinergic drugs ( $F=1.02$ ;  $P=0.396$ ). Diet type significantly effected AE2% ( $F=21.51$ ;  $P=0.001$ ). The two techniques ('ratio' and gravimetric) used for estimating assimilation efficiency showed a significant difference of 7% ( $F=16.73$ ;  $P=0.001$ ).

The dry weight of prawn was virtually 25% of wet weight (WW) and wet weight of prawns with time (days) was best estimated by the following quadratic relationship:

$$WW = 0.081(\text{Time}) + 0.0016(\text{Time}^2) - 0.97$$

Using the model:  $I = G + Ev + M + E$ , the energy budget for the total development of juvenile P.monodon was:

$$1175.8 = 341.4 + 29.6 + 227.3 + 577.5 \text{ (KJ)}$$

from which the following efficiencies were calculated; assimilation efficiency = 50.8%, gross growth efficiency (K1) = 21.9% and net growth efficiency (K2) = 43.0%.

## Introduction

Interest in decapod nutrition and digestion has been stimulated by the recent increase in commercial prawn farming. Whilst dietary requirements of juvenile penaeids are well known (Kanazawa et al., 1971; Cowey and Forster, 1971; Balazs et al., 1973; Sick and Andrews, 1973; Colvin, 1976; Sedgewick, 1979), little information is available on the feeding biology of penaeids. Knowledge of feeding behaviour of prawns in culture is particularly important in the design of artificial feeds and feeding techniques to economically replace current live feeds.

Additionally, accurate knowledge of the quantity of feed required for a given wet weight of prawn and an appreciation of the digestive capabilities of captive prawns are a prerequisite for optimising the conversion of feed to marketable flesh. Knowledge of ingestion rates can reduce feed costs and prevent over feeding, water quality deterioration and restricted growth through inadequate ration.

The present work investigates the effect of the wet weight (WW) of prawn, different feeds, and percentage body weight ingested on the assimilation efficiency and gut evacuation time of Penaeus monodon. A bioenergetic model is presented for P.monodon from 0.097g to 41.45g wet weight, grown on a dry pelleted diet. Furthermore, with a view to increased efficiency and optimal rate of food utilisation, the effect of cholinergic drugs on gastro-intestinal activity of the prawn is also investigated, with particular reference to artificial diets (Fange and Grove, 1979).

## Materials and methods

Penaeus monodon (Fabricius) postlarvae were obtained from Unilever Research (Findon, U.K.) and from Frippak feeds (Findon, U.K.). Gut evacuation time (GET) and assimilation efficiency (AE%) were investigated by collecting faeces at hourly intervals from prawns of differing sizes, previously starved for 24h. Because of the inherent difficulties of using Chromic oxide to estimate assimilation efficiencies in prawns (Foster and Gabbott, 1971) we estimated assimilation efficiency (AE%) in P.monodon using both the gravimetric :  $AE1\% = [(I-E)/I] \times 100$  (Crisp, 1984), and the "ratio" method, AE2% (Conover, 1966a,b; Condrey et al., 1972). Each group was kept in  $0.5m^2$  enclosures and fed a known dry weight of food. Collected faecal material was dried in an oven at  $60^\circ C$  for 24h to give the percentage ingested material defaecated with time (%IMF) and then pooled to assess the daily total of ingested material defaecated, from which AE1% was derived. Pooled samples were subsequently ashed at  $500^\circ C$  (Parsons et al., 1984) to give the assimilation efficiency (AE2%) following the 'ratio' method. GET (h) and assimilation efficiency were estimated for groups of prawns fed on different diets and levels of food (see Table 1). To establish the effects, if any, of drugs on GET and assimilation efficiency, L-glutamic acid (M.W=169.1:  $1.274 \text{ g L}^{-1}$ ), 5-hydroxytryptamine (M.W=387.4:  $3.233 \times 10^{-3} \text{ g L}^{-1}$ ) and carbachol (M.W=182.65:  $0.378 \text{ g L}^{-1}$ ) were prepared in seawater at concentrations an order of magnitude below incipient irritant levels following Prosser et al., (1965).

To determine growth rates, prawns were reared in tanks of volume  $4-12\text{m}^3$ , without an earth substratum, at an initial density of  $20\text{ m}^{-2}$  for the first 25 days and  $8\text{ m}^{-2}$  thereafter at 32.5‰,  $28^\circ\text{C}$  in a 12h light/dark regime. Prawns were fed 3-4 times daily on Findon pellets (FD: Frippak Feeds U.K. Ltd). Faeces, moults and excess food were removed daily. Total length (cm), carapace length (cm) and wet weight (g) of a sample of prawns were measured each day for the first 25 days and weekly thereafter. Dry weights were obtained after drying prawns in an oven at  $60^\circ\text{C}$  for 24h.

The energy budget for P.monodon can be summarised by the formula:

$$I = G + E_v + M + E$$

where I is the energy intake, estimated from the ingestion rate, given by the difference between dry weight of feed offered and dry weight of feed uneaten. G is the growth energy,  $E_v$  is the energy content of the exuvia and M is the metabolic energy expenditure estimated by oxygen uptake (for details see Kurmaly et al., 1989a). Oxygen consumption was measured 1-2h after feeding. Excretion and egestion E, were not determined but derived from the difference between assimilated energy ( $G+E_v+M$ ) and ingested energy (I). Energy contents were obtained using a Gallenkamp ballistic bomb calorimeter (Model: CB 330) (Paine, 1964, Phillipson, 1964). Assimilation efficiency (AE%; by energy), gross growth (K1) and net growth efficiency (K2) were also determined.

## Results

To standardise errors from determinations of the percentage of ingested material defaecated (%IMF) with time and thus facilitate comparison, the time required for 50% of the faeces to be evacuated from the gut (GET) was used to examine the effect of various parameters on %IMF. GET (derived from the log/log relationship between percentage of ingested material defaecated and time(h)), although affected by diet type, percentage body weight ingested (%BWI) and wet weight of prawn (Table 2), appears to be unaffected by cholinergic drugs (Table 3). The increase in time taken to ingest larger quantities of food obviously accounts for part of the increase in GET with %BWI.

Although assimilation efficiency (AE2%) is independent of %BWI, wet weight of prawn (Table 4a,4b) and cholinergic drugs ( $F=1.02$ ;  $P=0.396$  :Table 5a), it is significantly affected by diet type ( $F=21.51$ ;  $P=0.001$  :Tables 5a and 5b). Prawns fed on a diet of mussel or a diet incorporating Kaolin as a filler (Tables 5a and 5b), showed significantly higher (80%) and lower (55.44%) efficiencies respectively. The use of pre-starved prawns had no affect on assimilation efficiency. The absence of a significant difference between the assimilation efficiency of starved and non-starved prawns (Table 5a), can be explained by the duration of the secondary digestive process which lasts for up to 24-48h (Al-Mohanna, 1985 a,b; Al-Mohanna and Nott, 1987), indicating that a longer starvation time is required for a difference to be evident. Figure 1 is a plot of the percentage rate of material defaecated (%IMF) over 24h and shows that a constant

proportion of foregut content is defaecated per unit time. Irrespective of the amount defaecated with time, assimilation efficiency fluctuates between 60 to 80% for prawns of between 0.08g WW and 40.6g WW (Figure 2).

The two techniques used for estimating assimilation efficiency ('Ratio'  $x=70.89\%$ ; Gravimetric  $x=77.66\%$ ) were compared for a group of prawns ( $n=68$ ), between 0.08g and 40.6g WW, and were shown to differ significantly ( $F=16.73$ ;  $P=0.001$ ). The difference between the means was  $6.77\%(+0.862 \text{ S.E.})$ .

Table 6 describes relationships between various parameters necessary to estimate specific energetic relationships for P.monodon fed on artificial diets. Dry weight (equation 1, Table 6) is virtually 25% of wet weight; the exponent of the power relationship is not significantly different from 1. Both carapace and total length (equations 2 and 3, Table 6) are related to wet weight through simple power relationships with the exponent in each case being close to 3. The wet weight of prawns with time is best estimated by use of a quadratic relationship (equation 4, Table 6). The growth of prawns during the observations was essentially exponential for the first 25 days ( $WW= 0.09e^{0.1T}$ ,  $r^2= 0.99$ ). After this time the prawns were reared at a lower density ( $10 \text{ m}^{-2}$ ) and growth remained linear ( $WW=0.32T-8.1$ ,  $r^2=0.99$ ). Estimated growth rate at 25 days is approximately  $0.11\text{g day}^{-1}$  (wet weight) compared to the constant rate of  $0.32\text{g day}^{-1}$  found after the transition to larger tanks.

The daily energy budget for P.monodon, up to 40g wet weight, is presented in Table 7. The weight of ingested material increases with body weight (equation 5, Table 6) however, the logarithmic nature of the relationship shows that larger prawns eat less per g body weight than smaller prawns. Indeed, the percentage body weight ingested each day is a reciprocal function of body weight (equation 6, Table 6) decreasing from some 40-45% for early postlarvae to a mere 5-6% for late juveniles.

Assimilation efficiency (AE3%) calculated from the energy budget (Table 8a) bears no relationship to wet weight and fluctuates between 47-65%. Both gross growth and net growth efficiency show initial increases with prawn wet weight, up to 0.99g, and thereafter decrease. Thus for smaller prawns more energy is directed towards growth than metabolism whereas the converse is true for larger prawns. Such a change may be consequent upon the switch from an exponential to a linear growth rate.

## Discussion

The results show that prawns were able to fill their foreguts to 60-70% of capacity within 6-15 min and that smaller prawns had a faster ingestion and defaecation rate than large prawns (Table 2), which is in agreement with the higher metabolic and growth rate attributed to smaller prawns (Sick et al., 1970; Heyraud, 1979; Haddon and Wear, 1987). The timing of enzyme release of within 15 min of feeding (Barker and Gibson, 1977) is consistent with a rapid digestion rate and release of faecal material within 2-4h of feeding (Table 2).

The lack of correlation between assimilation efficiency and the amount of feed egested with time (Figure 2), suggests that there is no storage of indigestible material and that particles of FD diet were readily digestible and uniformly distributed in the pellet. The absence of varying assimilation efficiency with dry weight of meal ingested and prawn wet weight (Table 4a,4b) indicates that the ratio between enzyme concentration and food quantity remains virtually constant. Smith et al., (1985) working with P.vannamei have also reported assimilation efficiencies independent of prawn size. The constancy of assimilation efficiency with %BWI (Table 4a,4b) suggests that with each additional package of food ingested more enzymes are released, and that gastric evacuation of a homogeneous readily digestible diet does not proceed independently of gastric digestion, nor is it related to food availability. Consequently, unlike zooplanktonic organisms where food availability determines ingestion rate which in turn affects

the GET (Murtaugh, 1984, 1985; Kurmaly et al., 1989a), in juvenile prawns it is the digestion rate that determines ingestion and hence defaecation rate. In this respect prawns can be viewed as conservative feeders, ingesting no-more than can be effectively assimilated at one time. Feedback mechanisms controlling satiation in crustacea are discussed by Gelperin (1971), Barton-Browne et al., (1975) and Robertson and Laverack (1978). Return of appetite was detected in most prawns within 1-2h of feeding, once food entered the midgut, and is therefore related to foregut rather than complete intestinal emptying.

Models of optimal foraging argue that animals should be capable of adjusting gut passage time depending on food conditions (Lehman, 1976; Taghon, 1980). The presence of a feedback control mechanism within the foregut of organisms accounting for the constant nature of assimilation efficiency with %BWI is an assumption of the optimal digestion theory (Penry and Jumars, 1986, 1987). Such a mechanism has been reported for the spiny lobster Panulirus interruptus by Hartline et al., (1979a,b) where a sensory unit within the duct of the hepatopancreas was shown to excite monosynaptically the AB/PD neurones accelerating the pyloric rhythm. The presence of sensory neurones in the gut could confer plasticity to rhythmic behaviour such that feeds of varying quality and digestibility are treated differently. This mechanism is essential for an omnivorous feeder with reportedly wide tastes (Marte, 1980; Chong and Sasekumar, 1981).

Since it is likely that sensory neuromuscular synapses within the decapod foregut (Atwood, 1982) control the mechanism regulating digestive rate the effect of various drugs on digestion rate and food conversion efficiency was measured. Although more work is required, present results indicate that concentrations of the drugs employed in seawater do not effect the rate of gastro-intestinal activity (Table 3) and hence food utilisation in penaeids (Table 5a). Recent pharmacological work however, with high drug concentrations in seawater and ionotrophic applications of ACh ( $2-5 \times 10^{-5} \text{M}$ ) with  $10 \text{g L}^{-1}$  Tenisilon have produced contractions and depolarisations in the dorsal and ventral dilator muscles of the foregut (Marder, 1976). While ACh figured as a transmitter in the foregut, activating strong extrinsic muscles, L-Glutamate excited weaker intrinsic muscles, and then only at high concentrations ( $5 \times 10^{-4} \text{M}$  to  $10^{-3} \text{M}$ ) (Lingle, 1980; Atwood, 1982). It is likely, given the correct drug and concentration within a diet that foregut activity can be influenced by cholinergic drugs.

Assuming that the pattern of defeacation reflects the mechanics of the foregut, gastric digestion of newly ingested food can be divided into two stages. There is an initial delay while gastric juices penetrate and start to act on the food, and an emptying phase, dependent on dry weight of food and food quality in different and similar sized prawns (Table 2). A compact, dry pelleted diet (e.g. FD pellet) passes quicker through the gut than a diet of mussel; giving a low GET (Table 2) and AE% (Table 5a, 5b). Similar results have been shown for P. japonicus (Nose, 1964), Palaemon varians (Snow, 1969) and Metapenaeus benettiae (Moriarty, 1976).

Preliminary results of GET for prawns fed on mussel are comparable to those reported for Gammarus pulex (Martin, 1965), M.bennattae (Dall, 1967), Scylla serrata (Hill, 1976), P.monodon (Marte, 1980) and P.semisulcatus (Al-Mohanna and Nott, 1987) fed on soft foods.

The growth rates of P.monodon (equations 6 and 7 Table 6) fed with FD pellets, are comparable to those of penaeids in grow-out ponds fed on a combination of artificial and natural diets (Table 9). The variability in laboratory growth rates (Table 9), is attributable to the different species used (Liao et al., 1983), percentage composition of the diet, particularly protein (Kanazawa et al., 1970; Lee, 1971), protein quality (Smith et al., 1985), dietary energy content (Sedgewick, 1979a), diet stability in water (Bages and Sloane, 1981) and the stock density (Cuzon et al., 1982). Additionally, increased feed frequency, resulting in a more effective utilisation of diet, has been shown to improve growth rates (Sedgewick, 1979).

Despite the limitations inherent in estimates of biological energy flow (Kurmaly et al., 1989a), measurements were obtained for the major parameters (ingestion, egestion and excretion, growth and metabolism) involved in energy flow through P.monodon. Energy loss due to moulting is negligible (Table 7) and the daily percentage body weight ingested ( $\%BWI\ day^{-1}$ ) of 43.3% corresponds closely to the maximum ration of 48% found for prawns of approximately 0.1g WW, when fed on a pelleted diet (Sedgewick, 1979).

The energy budgets reveal that a greater portion of assimilated energy appears to be used for growth rather than metabolism in prawns up to 5.0g WW. This is in contrast to the partitioning of assimilated energy in several crustaceans; the isopod Idotea baltica (Tsikhon-Lukanina and Lukasheva, 1970; Strong and Daborn, 1979), the euphausiid Euphausia pacifica (Lasker, 1966), the deposit feeding amphipod Hyallela azteca (Hargrave, 1977) and the lobster Homarus americanus (Logan and Epifanio, 1978), where metabolic energy expenditure accounts for over 50% of assimilated energy. This ability in P.monodon, to channel a significant portion of ingested energy into growth accounts for the remarkably rapid growth (Wickins, 1980).

The daily and cumulative assimilation efficiencies calculated from the energy budget (Tables 8a, 8b) are generally lower than those reported by Condrey et al., (1972) for P.setiferus and P.aztecus (54-87%) and by Forster and Gabbott (1971) for Palaemon serratus (72-97%) calculated using the 'ratio' and gravimetric method respectively. Although dry pelleted feeds have been reported to be more poorly utilised than fresh or moist diets by crustaceans and fish (New, 1976; Storebakkan, 1985), it is unlikely that the low assimilation efficiency in this study is related to diet digestibility, since values as high as 85% have been achieved gravimetrically (Table 4a). Consequently the difference is most likely attributable to the choice of method used to estimate AE%. Additionally, as oxygen consumption was measured over periods of short duration and not continuously over 24h, which in this instance takes into account standard metabolism and the cost of digestion (SDA), results may not be representative of true

daily respiratory rates in culture conditions. To the extent that the loss of energy by this route is underestimated, the estimate of assimilation efficiency is likewise affected. A more precise measurement of metabolic energy loss would take into account the unknown cost of feeding and swimming. Considering the high level of swimming activity shown by the prawns in captivity, possibly reminiscent of their migratory behaviour during this period in the wild (Liao et al., 1983) and the ingestion rates demonstrated, feeding and swimming costs could be high enough to raise the assimilation efficiency further (Heyraud, 1979; Bishop et al., 1980; Dall, 1986).

Comparison between fed metabolism and standard metabolism, given by Kurmaly et al., (1989a) for P.monodon, shows that the differences between the two decreases from 54% to 14% with increase in dry weight, indicating that the standard metabolism in large prawns differs little from fed metabolism, possibly due to an increased digestion efficiency. Similar differences between fed and standard metabolism with dry weight have been reported for Carcinus maenus (Wallace, 1973), H.americanus (Logan and Epifanio, 1978; Capuzzo and Lancaster, 1979) and Meganytiphanes norvegica (Heyraud, 1979).

Gross growth efficiency (Table 8a) follows a similar pattern to that shown by H.americanus (Logan and Epifanio, 1978); an increase during exponential growth (0.097-0.99g WW) and a decrease during linear growth (0.99-52.78g WW). The cumulative gross growth efficiency (Table 8b) reported here is substantailly higher than those reported for I.baltica (3.4%) and Sphaeroma pulchellum (5.1%) (Tsikhon-Lukanina and

Lukasheva, 1970). Possibly, dissimilar life history strategies account for the difference (Barnes and Hughes, 1982). Decreasing net growth efficiency (Table 8a) with increase in dry weight has also been shown for the amphipod Pontogammarus maeoticus (Soldatova, 1970) and is a reflection of the disproportionate increase in maintenance costs with dry weight (Wolverkamp and Waterman, 1960), due to the surface area/volume effect.

Table 1. Proximate composition (%) of natural and artificial diets and their energy content (Jmg<sup>-1</sup>).

	Protein	Fat	Carbohydrate	Ash	Energy
Mussel*1	57.2	4.6	20.4	8.6	20.86
FD pellet	45.5	9.0	30.0	15.5	22.02
Sal*2	50.5	16.2	23.3	9.0	20.66
Soya*3	48.8	11.2	26.5	10.7	19.06

\*1 Sedgewick (1979a)

\*2 ground salmon feed with 10% wheat gluten.

\*3 Soya meal (55%), Krill meal (25%), Vitamin mix (2%),

Cod liver oil (8%) and wheat gluten (10%).

Table 2. The linear relationship ( $GET = a + bx(\%BW)$ ) between time for defaecation of 50% of meal (GET) and the meal size (%BW) for prawns of different sizes. S.E of the coefficient in parenthesis. The time for 50% GET for prawns fed different diets of 8 %BW is also given.

Prawn wet weight (g)	Diet type	Range (%BWI)	N	a	b	r2	P	50% GET for 8 %BWI*1.
0.134	FD	4-220	12	2.61	0.042	0.773	0.001	-
0.227	FD	13-60	9	2.18	0.089	0.783	0.001	-
6.09	FD	8-40	9	3.02	0.086	0.817	0.001	3.7 5.4 6.6 3.9
40.6	FD	2-8	9	4.09	0.513	0.939	0.001	8.2 - 8.4

**\*1 mean of 10.**

**\*2 FD with 30% Kaolin as filler.**

**\*3 Mussel**

\*4 starved for 48h and fed FD pellets.

Table 3. The effect of cholinergic drugs; L-glutamic acid (GLA), 5-hydroxytryptamine (5HT) and carbachol(CARB) on the gut evacuation time (GET) of prawns (6.09g wet weight).

Number of prawns	Drug	Concentration (g L <sup>-1</sup> )	%BWI	Time (h) for 50% GET (+S.E)	
					*1
12	GLA	1.274	15	4.07	(0.270)
10	5HT	0.003	15	4.09	(0.224)
10	CARB	0.378	15	4.25	(0.189)
10	CONTROL	----	15	4.34	(0.210)

Results of oneway anova on GET50%:

\*1 F=0.30; P=0.828 not significant at the 5% level.

Table 4a. Mean assimilation efficiencies (AE2%) estimated by the 'ratio' method (Conover, 1966a,b) for prawns fed meal sizes in the range 3-220 of percentage body weight (%BW). Number of prawns tested in parentheses.

Prawn wet weight (g)	Percentage body weight ingested (%BW)									
	4-18	18-25	25-40	40-50	50-60	60-70	70-80	80-100	100-220	
0.077	72.39 (5)	83.49 (3)	70.48 (5)	60.48 (7)	78.52 (3)	60.54 (5)	-	-	-	
0.130	74.38 (3)	67.24 (2)	68.92 (5)	58.69 (5)	62.68 (3)	70.72 (3)	69.03 (5)	59.39 (3)	-	
0.215	70.98 (2)	61.35 (2)	63.20 (4)	71.48 (6)	73.54 (3)	66.58 (2)	66.35 (5)	59.02 (3)	-	
1.120	61.35 (3)	56.95 (3)	49.69 (2)	58.20 (5)	64.72 (7)	65.90 (4)	67.41 (4)	58.35 (5)	70.45 (3)	
6.09	69.73 (4)	61.84 (6)	62.97 (3)	67.35 (6)	68.52 (6)	66.53 (6)	70.42 (6)	71.27 (5)	64.64 (6)	

Table 4b. Two factor analysis of variance on assimilation efficiencies estimated by the 'ratio' method for P.monodon (0.077g-6.09g WW) fed different rations in the range 3-220 of percentage body weight (%BWI).

Source of variation	Sum of Squares	DF	Mean Square	F	P
Wet weight	937.103	4	234.276	1.443	0.223
Meal size	996.891	8	124.611	0.768	0.632
Interaction	3644.488	27	134.981	0.832	0.704
Residuals	20774.398	128	162.300		
Total	26271.712	167	157.316		

Table 5a. The effect of cholinergic drugs; L-glutamic acid (GLA), 5-hydroxytryptamine (5HT), carbachol (CARB), diet type or pre-condition of prawns of 6.09g WW on the assimilation efficiencies determined gravimetrically (AE1%) and by the 'ratio' method (AE2%).

Diet type	Drug	N	%BWI	Mean AE2% 'Ratio' (+S.E) *3	Mean AE1% Gravimetric (+S.E)
FD	GLA	8	15	73.79 (3.06)	83.61 (2.21)
FD	5HT	10	15	63.57 (3.33)	85.63 (5.33)
FD	CARB	8	15	66.65 (4.59)	78.16 (5.74)
FD	CONTROL	12	15	63.61 (4.37)	84.36 (2.80)
<hr/>					
FD*1	--	27	8	*4 56.18 (2.75)	50.03 (1.77)
FD*2	--	29	8	73.60 (2.05)	77.85 (0.735)
FD	--	16	8	64.41 (1.76)	76.45 (1.51)
Mussel	—	16	8	80.87 (1.97)	89.87 (1.37)

\*1 FD with 30 % Kaolin as a filler.  
 \*2 pre-starved 24h .

Oneway analysis of variance:  
 \*3 F=1.02; P=0.396 no significant difference at the 5% level.  
 \*4 F=21.51; P=0.001 significant at the 5% level.

Table 5b. Scheffe's test: + denotes pairs of groups in block 2 (above) which are significantly different at the 5% level. 95% C.I. for mean(i) - mean(j) is = 7.745 x Range x (1/n(j) + 1/n(i))<sup>0.5</sup>. Range for 0.05 level= 4.03

Mean		FD*1	FD	FD*2	Mussel
56.18	FD*1				
64.41	FD				
73.60	FD*2	+			
80.87	mussel	+	+		

Table 6. Relationships between various growth and feeding parameters for p.monodon (0.01-40 g WW) fed on a artificial diet.

Equation	n	95% CI of coefficients	r <sup>2</sup>
1 DW= 0.25(WW) <sup>1.01</sup>	50	0.0094 (WW)	0.95
2 WW= 0.51(CL) <sup>2.95</sup>	155	0.0051 (CL)	0.98
3 WW= 0.0074(TL) <sup>2.81</sup>	149	0.0058 (TL)	0.97
4 WW= 0.081(T)+0.0016(T <sup>2</sup> )-0.97	41	0.026 (T) 0.00022 (T <sup>2</sup> )	0.99
5 IR= 0.0645(WW) <sup>0.62</sup>	7	0.151 (WW)	0.95
6 %BWI= 1/(0.0254 +0.0045WW)	7	0.0006 (WW)	0.98

Where DW= dry weight (g), WW= wet weight (g), CL= carapace length (cm), TL= total length (cm), T= time (days), %BWI= % of body weight ingested daily, IR= ingestion rate (g dry weight animal<sup>-1</sup> day<sup>-1</sup>).

Table 7. Daily energy budget for juvenile P.monodon fed on FD pellets. Energy content of prawn given by 1g dry weight=20.76 KJ, ingestion (I) assuming 1g food =22.02 KJ, metabolism (M) given assuming 1mg O<sub>2</sub>= 14.06 J (Gnaiger, 1983). Exuvia (Ev) assuming 1g moult= 6.68 KJ, growth (G) assuming 1g dry weight= 20.76 KJ and egestion/ excretion (E) given as difference I-(M+G+Ev).

Time (days)	Wet weight (g)	Energy content of prawn (KJ)	%BWI	I (J)	M (J)	Ev (J)	G (J)	E (J)
0	0.097	0.49	43.3	220.1	28.41	17.97	82.30	91.42
13	0.304	1.56	40.0	660.6	68.99	39.62	290.00	261.99
25	0.987	5.04	42.0	2193.85	174.75	154.44	1099.47	765.19
44	5.075	25.93	20.4	5549.1	700.94	173.31	1768.5	2906.3
84	18.92	96.67	8.0	8311.68	2139.43	229.2	1763.14	4179.91
119	30.99	158.38	6.0	10047.0	3172.76	200.36	1669.68	5004.23
151	41.45	211.81	5.0	10971.5	4012.73	241.75	1490.31	5226.71

Table 8a. Daily efficiencies of P.monodon juveniles fed on FD pellets derived from the energy budget.

Wet weight (g)	Assimilation efficiency (AE3%)	Gross growth efficiency (K1%)	Net growth efficiency (K2%)
0.097	58.46	45.56	77.92
0.304	60.34	49.89	82.69
0.987	65.12	57.15	87.76
5.075	47.67	34.99	73.47
18.92	49.71	23.97	48.22
30.99	50.19	18.61	37.08
41.45	52.36	15.19	30.15

Table 8b.Cumulative energy budget and efficiency for the development of P.monodon juveniles (0.097g WW to 41.45g WW) calculated from Table 2.

I	M	Ev	G	E	AE3	K1	K2
(KJ)	(KJ)	(KJ)	(KJ)	(KJ)	%	%	%
1175.77	341.37	29.59	227.30	577.51	50.86	21.85	42.94

Table 9. Comparison of penaeid growth rates and feed conversion ratios (FCR); g dry weight of feed required to produce g dry weight of prawn.

Species	Initial-final wet weight g WW	Density m	Diet	Growth rate g WW day	FCR	Author
<u>P.japonicus</u>	5.3-15.7	89	Pellet	0.173	1.3	Deshimaru + Shigeno(1972)
<u>P.japonicus</u>	2.5-6.3	30	Pellet	0.031		Cuzon et al., (1982)
<u>P.japonicus</u>	7.0-12.9	6.5	Pellet	0.097		"
<u>P.merquensis</u>	0.3-3.4	10.3	Mussel	0.055	5.1	Sedgewick (1979)
<u>P.merquensis</u>	0.3-3.2	10.3	Pellet	0.051	3.27	"
<u>P.monodon</u>	0.02-0.037	552	Pellet	0.00064	4.94	Bages and Sloane(1981)
<u>P.monodon</u>	<2.0	25	Trash + Pellet	0.211*1	1.2-3.3	Liao et al (1983)
<u>P.monodon</u>	0.097-41.5	8/20	FD Pellet	0.284	1.28	Present work
<u>P.vannamei</u>	4.0-9.7	6.6	Pellet(36A)	0.210		Smith et al., (1985)
<u>P.vannamei</u>	9.8-14.5	6.6	Pellet(36A)	0.190		"
<u>P.vannamei</u>	20.8-24.0	4.4	Pellet(36A)	0.120		"

\*1 reared in grow-out ponds

Figure 1. Logarithmic curve of the percentage of ingested material faecated (IMF) against time (h) for prawns of 0.097g WW fed 21.44% body weight (BW). Relationship:

$$\log(\%IMF) = 1.14 + 0.680 \log (\text{Time}).$$

For all prawns tested 0.097g to 41.45g WW fed 3 to 80% body weight, b (slope) ranged from 0.3 to 1.0.

Figure 2. Percentage assimilation efficiency against percentage material faecated over 24h for prawns of between 0.077g WW to 40.6g WW fed 30 to 40% of body weight.

Figure 1

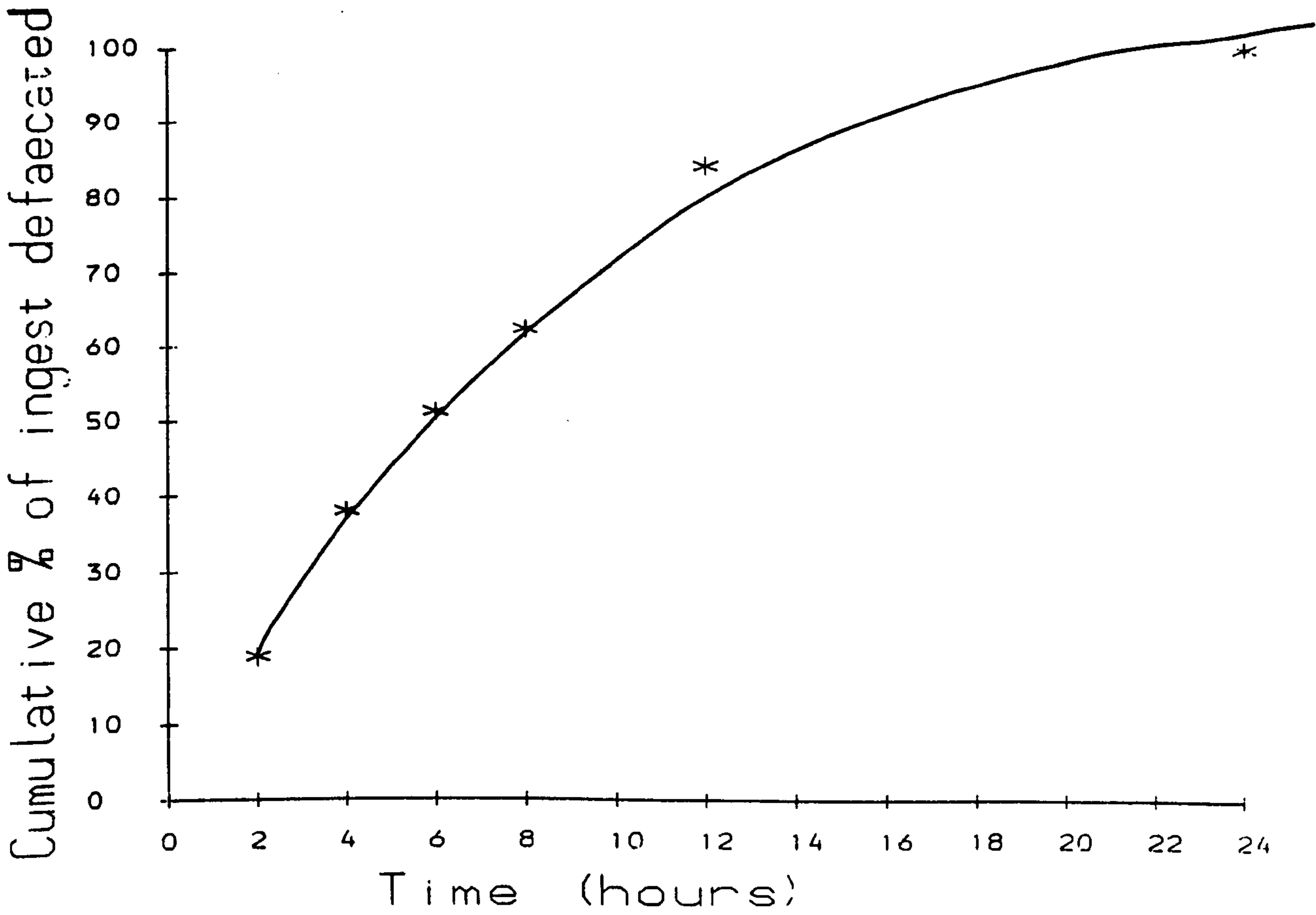
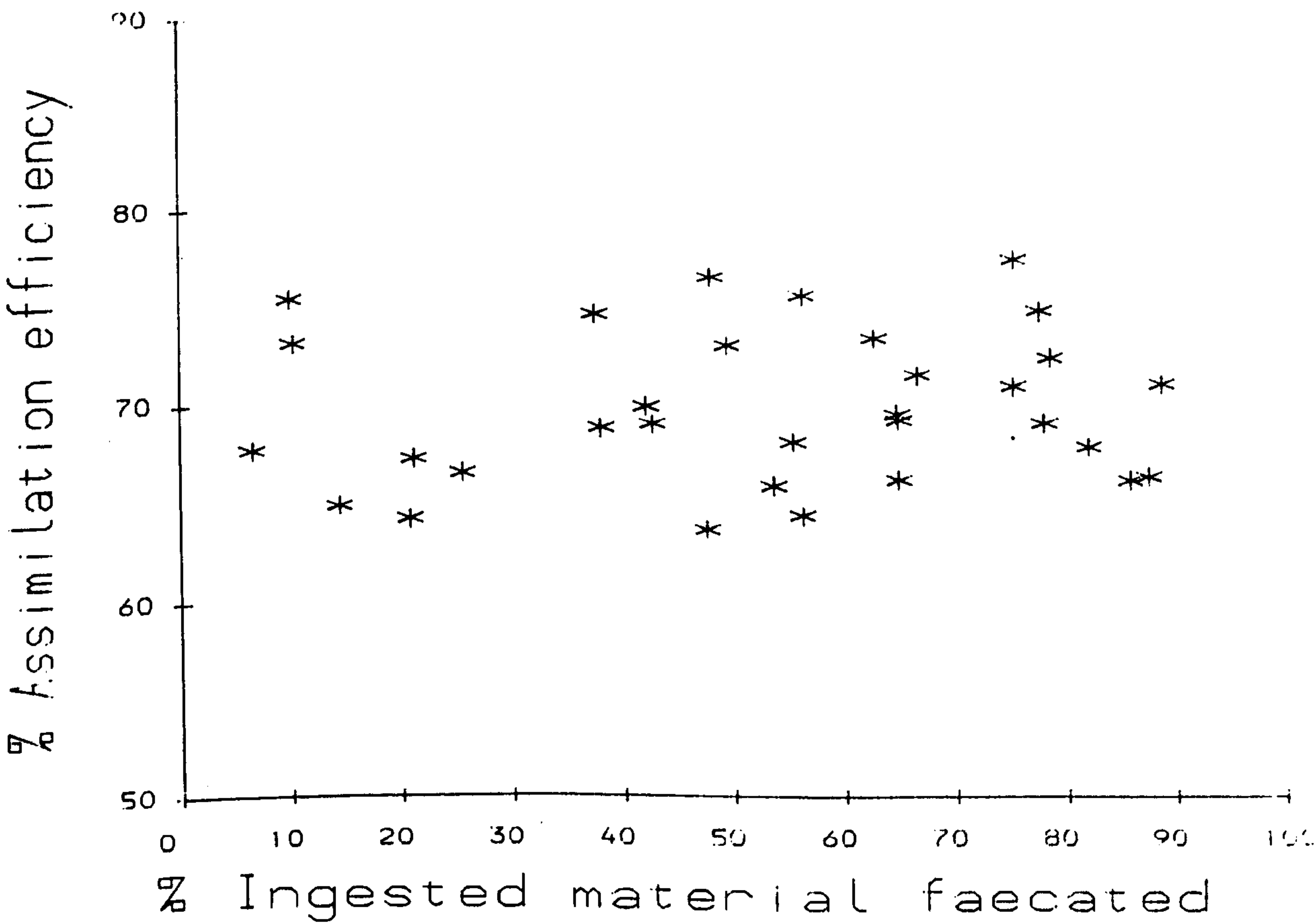


Figure 2



Physiological energetics of the larval development of Homarus  
gammarus (Linnaeus).

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## Abstract.

Carapace length (C.L. mm) increased logarithmically with time (T:days) and fitted the following function:

$$C.L. = 2.52x (T)^{0.234}, r^2 = 0.92 .$$

The curvilinear relationship between carapace length and dry weight (DW:mg) given by the geometric mean regression of semi-log transformed data was:

$$DW = 0.32x(10^{0.296xC.L.})$$

Ingestion rate ( $\text{mg L}^{-1} \text{d}^{-1}$ ) on a mysid diet increased from stage I to V. Weight specific ingestion rate ( $\text{mg mg}^{-1} \text{d}^{-1}$ ) decreased with stage except for stage III. The relationship between oxygen consumption ( $\mu\text{l O}_2 \text{ L}^{-1} \text{h}^{-1}$ ) and dry weight (DW:mg) for the larval stages I to V, using geometric mean regression analysis was:

$$\mu\text{l O}_2 \text{ L}^{-1} \text{d}^{-1} = 3.47x (DW)^{0.87}$$

Using the model:  $I = G + Ev + M + E$ , the daily energy budget for the total development of H.gammarus larva was:

$$334.7 = 145.6 + 45.7 + 162.9 + 69.9 \text{ (Joules)}$$

With the exception of stage II and IV, energy incorporated into growth exceeds metabolic energy loss and accounts for between 25% to 38% of ingested energy. Daily efficiencies for individual stages (I-V) calculated from the energy budget are as follows; assimilation efficiency = 79%, gross growth efficiency (K1%) = 57% and net growth efficiency (K2%) = 54% .

## INTRODUCTION

As the primary aims of mass cultivation of commercially important decapod species are to maximise survival and growth, particularly in intensive culture systems where input costs are high, information on energy budgets, feed transfer efficiency, feed quality and optimal feed regimes are essential. In contrast to the practical utilisation of energy budgets in the farming of poultry and ruminants where practical use of energy budgets in cost-benefit analyses has helped in efficient resource management (MAFF, 1974, 1975), bioenergetic models have not yet been widely adopted in aquaculture.

Published studies on the physiological energetics of decapods rarely include an account of energy flows through planktonic decapod larval stages where much reproductive energy is lost in high mortality (Reeve, 1969; Mootz and Epifanio, 1974; Logan and Epifanio, 1978; Johns, 1982).

This study presents an energy budget for all the larval stages of Homarus gammarus fed on frozen mysids and discusses the effect of different quality feeds on the growth rate of lobster larvae. The differences between energy budgets of various decapod larvae are discussed relative to their feeding and life strategies.

## MATERIALS AND METHODS

Lobster larvae obtained from the North Western and North Wales Sea Fisheries Committee were reared individually in mesh bottom compartments of 5x5x5cm, each continuously supplied with filtered (<10  $\mu$ m) seawater (S=32.5‰ ; T=20°C) in a 12h Light:Dark regime. Larvae were fed twice daily after removal of faeces and waste food. Every two days the carapace length (mm) and dry weight (mg) of a sample of larvae (n= >14) was measured. Samples were washed in 0.9% ammonium formate dried at 60°C to constant weight, allowed to cool in a dessicator and weighed on an electrobalance (Mettler H54 AR).

Daily ingestion rates of lobster larvae were determined from the difference between the dry weight (DW) of feed given and dry weight of feed remaining, collected with a pipette under a binocular microscope, after 2-3h. Ingestion rates of individual larvae were determined in vessels of 250ml over a period of between 48-60h. For each stage the ingestion rate of between 10-20 larvae was determined. Ingestion rate is given by the following equation:

$$Ir = \frac{(Dg - Dr)n_1 + (Dg - Dr)n_2 + \text{etc}}{\text{time (hours)}}$$

where Dg = diet given and Dr = diet remaining. Fresh feed was offered every 2-3h after collection of previously uneaten feed. Preliminary trials showed that leaching of nutrients from the diet never exceeded 15% of dry weight after 4h in seawater. Egested and exuvial material were washed, dried and weighed as above.

Calorific measurements were made on an Gallenkamp ballistic bomb calorimeter (Model: CB 330) (Phillipson, 1964). Samples were dried as above and diluted with calorific benzoic acid standard for increased accuracy with small samples. Calories were converted to joules by assuming 4.18 Joules = 1 Calorie (Crisp, 1984).

For each stage routine respiration rates were measured for larvae throughout the moult cycle (Aiken, 1973, 1980), at  $20^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ , using a polarographic oxygen electrode (Model:E5046, Radiometer, Copenhagen) in vessels of 10-50ml capacity. Oxygen uptake was converted to energy utilisation on the assumption that  $1\text{ml O}_2 = 20.08\text{J}$  (Gnaiger, 1983).

The daily energy budget for each larval stage was assembled using the standard formula;

$$I = G + E_v + M + E$$

Where I is energy intake, estimated from the ingestion rate and G is the growth rate, calculated from the difference between the individual energy content of adjacent stages ( $\text{J indiv}^{-1}$ ) and the duration of each stage.  $E_v$  is the energy content of the exuvia and M is the metabolic rate estimated by oxygen uptake. Excretion and egestion E, were not determined, this value was derived from the difference between assimilated energy A and the ingested energy I.

Assimilation (AE%), gross growth (K1%) and net growth efficiencies (K2%) for each larval stage were calculated using the following formulae;

(AE%) :	$((M+G+Ev)/I) \times 100$	Crisp (1984)
(K1%) :	$((G+Ev)/I) \times 100$	Conover (1968)
(K2%) :	$((G+Ev)/(G+Ev+M)) \times 100$	Calow (1977b)

## RESULTS

The carapace length (mm), dry weight (mg) and duration of each larval stage of H.gammarus are given in Table 1. The dry weights were 20% to 55% greater than those of H.americanus larvae (Logan and Epifanio, 1978; Capuzzo and Lancaster, 1979; Sasaki et al., 1986). Carapace length (C.L:mm) increased logarithmically with time (T:days) and using least squares regression analysis fitted the following function :

$$C.L = 2.517x(T)^{0.234} \dots\dots 1$$

$$S.E \text{ of coefficient} = 0.006, n=120, r^2=0.92$$

The curvilinear relationship between carapace length (C.L:mm) and dry weight (DW:mg) given by geometric mean regression (Ricker, 1973) of semi-log transformed data was:

$$DW = 0.319x(10^{0.296xC.L}) \dots\dots 2, S.E \text{ of coefficient} = 0.008, n=133.$$

Daily energy intake for the larvae of H.gammarus (stages I-V) fed on mysids and recently hatched lobster larvae (RHL) are given in Tables 2 and 3. For both diets ingestion rate increased from stage I to V. Weight specific ingestion rate (WSIR:  $mg \text{ } mg^{-1} \text{ day}^{-1}$ ) decreased with stage except for stage III larvae fed on mysids or recently hatched lobster larvae.

Measurements of "routine" (Vernberg, 1983) metabolic rates are given in Table 4. The relationship between mean oxygen consumption ( $\mu l \text{ } O_2 \text{ larva}^{-1} \text{ h}^{-1}$ ) and mean dry weight (DW:mg) for larval stages I-V, using geometric mean regression analysis (Ricker, 1973), was :

$$\mu l \text{ } O_2 \text{ larva}^{-1} \text{ h}^{-1} = 3.47x (DW)^{0.87} \dots\dots 3$$

$$S.E. = 0.014, n=109.$$

No attempt was made to quantify energy loss through excretion

since it has been shown to be negligible (Logan and Epifanio, 1978; Capuzzo and Lancaster, 1979; Johns, 1982). A daily energy budget for each larval stage is presented in Table 5. The proportion of ingested energy invested in respiration increases from 37% (stage 1) to 46% (stage II) falling to 33% at stage V. Exuvial energy loss accounts for between 4-14% of ingested energy. With the exception of stage II and IV, energy incorporated into growth exceeds metabolic energy loss and accounts for between 25% to 38% of ingested energy.

Table 6 shows the major energetic efficiencies for H.gammarus larval stages I to V. Three values for assimilation efficiency are presented, AE1% and AE2% have been calculated from the dry weights of ingestion and egestion given in Tables 2 and 3, and AE3% from the energy budget given in Table 5. The mean assimilation efficiency by weight, of the larval stages fed on RHL (92%) exceeds the value of the mean assimilation efficiency given for a diet of mysids (83%) ( $n=10$ ;  $F=69.2$ ;  $P=0.001$ ). The assimilation efficiency of lobster larvae fed on mysids calculated by weight, corresponds closely to the efficiency derived from the energy budget ( $n=10$ ;  $F=0.35$ ;  $P=0.569$ ). Both gross growth efficiency (K1%) and net growth efficiency (K2%), increase with size from 42% (stage I) to 49% (stage V) and 53% (stage 1) to 60% (stage V), respectively.

## Discussion

Whilst the larval growth rate of H.americanus best fits a semi-logarithmic curve (Logan and Epifanio, 1978; Capuzzo and Lancaster, 1979), the growth rate of larval H.gammarus best fits a log/log curve. This contrast reflects the different diets, ration size, density of organisms used during culture, effect of container volume on growth and the difference in hatch size between the two organisms (Gruffydd et al., 1977; Van Olst et al., 1980; Eagles et al., 1986). The effect that diets of different quality have on growth is clearly exemplified by comparing Tables 2 and 3; lobster larvae ingest more recently hatched lobster larvae (RHL) than mysids. However, since the increased ingestion rate on RHL does not result in a corresponding increase in growth rate (Table 3), it is likely that RHL are either nutritionally deficient or costly to handle (Sulkin, 1975 ; Pyke et al., 1968). Studies by Sulkin (1978) and Sulkin and Norman (1976) have demonstrated the importance of dietary quality on growth and development of decapod larvae, showing growth may be dependent upon satisfying nutritional requirements at specific periods during the growth cycle (Sulkin, 1978). Additionally, Sasaki et al., (1986) have suggested that differences in egg yolk deposits in stage I may account for growth variability. Both H.gammarus and H.americanus however, show a peak increase in growth rate during larval development. In the present study the greatest increase of 189% is shown between stages III-IV, similarly the larval stages of H.americanus showed highest growth rate between stages III to IV (Capuzzo and Lancaster, 1979; Sasaki et al., 1986) or IV-V (Logan and Epifanio, 1978).

In common with most decapod larvae, lobster ingestion rates ( $\text{mg L}^{-1} \text{ day}^{-1}$ ) increase with larval development from stage I to V with the most rapid increase occurring between stages III to IV, corresponding to the high growth rate over this period. Although the weight specific ingestion rate (WSIR:  $\text{mg mg}^{-1} \text{ day}^{-1}$ ) decreases from stage I to V (Table 2 and 3), stage III shows a substantial increase. A similar increase in WSIR with decapod larval development has been shown for Hyas araneus (Anger and Dawirs, 1982) and possibly reflects the transition of the larva from a planktonic to a benthic existence.

Routine respiration rates of H.gammarus larvae are within the range of values reported for H.americanus (Logan and Epifanio, 1978) but higher than those given for portunid or xanthid larvae (Dawirs, 1983; Mootz and Epifanio, 1974). The regression coefficient (equation 3) determined for H.gammarus (0.87) is within the range of values reported by Richman (1958), Mootz and Epifanio (1974), Logan and Epifanio (1978), Capuzzo and Lancaster (1979) and Stephenson and Knight (1980) for decapod larvae. The high slope value indicates that the rate of change of respiration rate is greater than the rate of change of body size.

Based on equation 3 rates of weight specific oxygen consumption (WSRR:  $\mu\text{l O}_2 \text{ mg}^{-1} \text{ h}^{-1}$ ) for larval stages of H.gammarus compare favourably to the larvae of Uca (Vernberg and Costlow, 1966), Menippe mercenaria (Mootz and Epifanio, 1974), H.americanus (Logan and Epifanio, 1978; Capuzzo and Lancaster, 1979), Cancer irroratus (Johns, 1982) and Carcinus maenus (Dawirs, 1983). However, variations in metabolic

activity associated with larval development differ from those given by Capuzzo and Lancaster (1979) for H.americanus where WSRR is shown to increase with each successive larval stage and only decrease at the postlarval stage. Present results are similar to the work of Logan and Epifanio (1978) and show a decrease in WSRR with successive larval stages. Factors associated with the decrease in metabolic rate with increasing size are the higher activity levels of the initial stages, the decrease in surface area to volume ratio and the disproportionate increase in tissue of low metabolic rate (chitin and lipid deposits) during late development (Prosser, 1973; Sassaki et al., 1986).

Analysis of daily energy utilisation by larval stages of H.gammarus (Table 5) demonstrate that the ratio between ingested energy channelled into growth and metabolic activity (G/M), ranges between 0.55 and 1.16. Whilst certain stages are more efficient in directing energy towards growth (stages I, III and V), the cumulative energy budget suggests little overall difference between the portion of energy converted into body tissue and that used for metabolic processes (Table 7). Although such data are not strictly comparable due to technical and physiological variations inherent in studies of this nature, it is obvious from Table 7 that the G/M ratios of carnivorous larvae are approximately 1, those of omnivorous larvae e.g P.monodon >1.5 and herbivorous larvae e.g Elminius modestus >3. Whilst more data is required, values of >1.5 appear to be correlated with species that possess naupliar stages. Carcinus maenus which was cultured at a high temperature of 18 °C and H.americanus which shows low routine metabolism (Table 4) in small respirometer vessels (15ml), do

not seem to fit the general trend.

Assimilation efficiencies for lobster larvae of between 77% and 93% fall well within the range of values for carnivorous larvae (Welch, 1968) and certain decapods (Logan and Epifanio, 1978; Johns, 1982), but are higher than those of R.harisii (45%: Levine and Sulkin, 1979), Carcinus maenus (22.9%: Dawirs, 1983) and P.monodon (16.3%: Kurmaly et al., 1989). Additionally, unlike the larval stages of R.harisii, P.monodon and to a degree C.maenus, assimilation efficiency does not increase with larval stage, but is relatively constant.

Gross growth efficiencies (K1%) of H.gammarus larval stages I to III (Table 6) are considerably higher than corresponding efficiencies for the larvae of M.mercenaria, H.americanus, R.harisii, Cancer irroratus and Carcinus maenus (Mootz and Epifanio, 1974; Logan and Epifanio, 1978; Levine and Sulkin, 1979; Johns, 1982; Dawirs, 1983) which were cultured at high densities. Gross growth efficiencies of later stages however, show no difference to values reported for the above larvae, increasing larval mortality with time presumably alleviates problems of density dependent growth. The gradual increase of K1% in the present study, during larval development, is possibly related to a reduction of the relative respiration rate or to an increase in enzyme efficiency (Calow, 1977a).

Calow (1977b) estimated the theoretical optimal net growth efficiency for growing hetetrophs to lie between 70% and 80%. Although the calculated K2% of 54.0% (Table 7) for the larval stages of H.gammarus falls far short of the

theoretical optimum, with the exception of the larvae of Carcinus maenus (K2% ;17.2%) and P.monodon (K2%; 68.6%), the range (Table 6) overlaps the K2% efficiencies of the other decapod larvae discussed above. This implies that the larvae of H.gammarus are able to grow less efficiently than larvae of P.monodon. The apparent increase in K2% with larval development which indicates that production of new tissue consumes an increasing proportion of energy, has similarly been noted by Mootz and Epifanio (1974).

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**Table 1. Meristic characteristics of H.gammarus, stage I-VI. Standard error and sample size are given in parentheses.**

Stage	Duration (days)	Age (days)	Dry weight (mg)	Carapace length (mm)
I	5	1	2.19 (0.051) (42)	2.65 (0.028) (42)
II	5	5	3.38 (0.051) (28)	3.62 (0.061) (28)
III	6	11	4.37 (0.220) (21)	4.02 (0.092) (21)
IV	8	17	8.27 (0.483) (18)	4.74 (0.019) (18)
V	10	25	13.33 (1.11) (20)	5.44 (0.072) (20)
VI	12	35	25.62 (1.24) (14)	6.13 (0.091) (14)

Table 2. Daily ingestion (as weight and energy assuming 1mg dry weight= 22.32J), weight specific ingestion and egestion rate (as dry weight of faeces) of Homarus gammarus, stage I-V fed on mysids. Standard errors are in parentheses. (Growth rate= 0.0875 mm day<sup>-1</sup>, S.E=0.007, n=63). n=3.

Stage	Ingestion of mysids (mg L <sup>-1</sup> day <sup>-1</sup> )	(J L <sup>-1</sup> day <sup>-1</sup> )	Weight specific ingestion rate (mg mg <sup>-1</sup> day <sup>-1</sup> )	Egestion (mg L <sup>-1</sup> day <sup>-1</sup> )
I	0.464(0.034)	10.36	0.211	0.104
II	0.476(0.023)	10.62	0.141	0.107
III	0.896(0.064)	20.00	0.205	0.184
IV	1.174(0.032)	26.21	0.142	0.234
V	1.700(0.022)	37.95	0.127	0.294

Table 3. Daily ingestion (as weight and energy assuming 1mg= 17.40J), weight specific ingestion and egestion rate (as dry weight of faeces) of H. gammarus, stages I-V fed on recently hatched lobster larvae (RHL). Standard errors are in parentheses. (Growth rate=0.0761 mm day<sup>-1</sup>, S.E=0.005, n=120)

Stage	Ingestion of RHL (mg L <sup>-1</sup> day <sup>-1</sup> )	Weight specific ingestion rate (mg mg <sup>-1</sup> day <sup>-1</sup> )	Egestion (mg L <sup>-1</sup> day <sup>-1</sup> )
I	1.050(0.035)	0.479	0.100
II	1.692(0.055)	0.500	0.131
III	3.193(0.120)	0.730	0.243
IV	3.361(0.207)	0.406	0.272
V	4.400(0.168)	0.330	0.296

Table 4. Routine metabolic rate ( $\mu\text{l O}_2 \text{ L}^{-1} \text{ h}^{-1}$ ) measurements of H. gammarus, stage I-V and a comparison of the daily metabolic energy loss (assuming  $1\text{ml O}_2 = 20.08 \text{ J}$ ) between H. gammarus and H. americanus. Standard error and sample size are given in parentheses.

Stage	Oxygen consumption of <u>H. gammarus</u> ( $\mu\text{l O}_2 \text{ L}^{-1} \text{ h}^{-1}$ )	Present work	Logan and Epifanio 1978	Capuzzo and Lancaster 1979	Sasaki et al 1986
		<u>H. gammarus</u>	<u>H. americanus</u>	<u>H. americanus</u>	<u>H. americanus</u>
I	7.895 (0.417) (41)	3.81	1.30	0.72	1.93
II	10.075 (0.373) (18)	4.86	1.91	1.56	3.07
III	14.293 (1.192) (25)	6.89	2.41	2.79	4.63
IV	20.293 (1.558) (16)	9.78	4.21	9.01	13.87
V	26.451 (2.924) (10)	12.75	7.22	8.57	--

Table 5. Daily energy budget for the larval development of Homarus gammarus. (all figures in joules individual<sup>-1</sup>). Ingested and metabolic energy have been obtained from Tables 2 and 4 respectively. Exuvial energy loss was calculated assuming  $1\text{mg} = 8.72\text{ J}$  (logan and Epifanio, 1978). Growth is given by the difference between the daily energy content of each stage divided by it's duration and egestion/ excretion is given by the difference between ingested energy (I) and assimilated energy (M+Ev+G).

Stage	Energy per stage	Ingestion on stage(I) larvae (I)	Metabolic rate (M)	Exuvia (Ev)	Growth (G)	Egestion / excretion
I	38.11	10.36	3.81	0.49	3.91	2.15
II	57.66	10.62	4.86	0.94	2.69	2.13
III	71.10	20.03	6.89	1.63	7.48	4.03
IV	115.93	26.21	9.78	3.60	8.47	2.98
V	183.69	37.95	12.75	4.11	14.77	6.32

Table 6. Daily efficiencies for individual stages (I-V) of H.gammarus. AE1 and AE2 have been calculated (by weight) from Tables 2 and 3 respectively. AE3 has been calculated (by energy) from Table 5.

Stage	Assimilation efficiency (AE1%) fed mysids	Assimilation efficiency (AE2%) fed lobsters (I)	Assimilation efficiency (AE3%)	Gross growth efficiency (K1%)	Net growth efficiency (K2%)
I	77.58	90.47	79.25	42.47	53.59
II	77.52	92.25	79.94	34.18	42.78
III	79.46	92.38	79.88	45.48	56.95
IV	80.06	91.91	88.63	46.05	55.23
V	82.71	93.27	83.35	49.75	59.68

Table 7. A comparison of the cumulative energy budgets of several types of crustacean larvae. I= ingested energy; G= energy invested into growth; Ev= energy of exuvia; M= metabolic energy loss; E= egested and excreted energy. G/M is the ratio between energy invested into growth and metabolism.

Family	Species	I	G	Ev	M	E	T	C	G/M	Author
Cancridae	<u>Cancer irroratus</u> "	21.3	5.8	0.2	5.3	10.0	15	1.09		Johns(1982)
		15.3	6.0	0.2	5.5	3.7	24	1.09		"
Portunidae										
	<u>Carcinus maenus</u>	28.3	0.9	0.2	5.3	21.8	18	0.16		Dawirs(1983)
Xanthidae	<u>Menippe mercenaria</u>	30.7	9.2	2.1	8.1	11.2	25	1.13		Mootz and Epifanio (1974)
										Levine and Sulkin (1979)
	<u>Rhithropanopeus harisii</u>	15.4	3.5	0.8	2.6	8.5	25	1.35		
Homaridae	<u>Homarus americanus</u>	399.7	138.5	26.5	76.3	70.95	20	1.81		Logan and Epifanio (1978)
		334.7	145.6	45.73	162.9	69.9	20	0.894		Present work
	<u>Homarus gammarus</u>									
Penaeeidae	<u>Penaeus monodon</u>	18.8	1.7	0.4	0.96	15.7	28	1.77		Kurmaly et al., (1989)
Balanidae	<u>Elminius modestus</u>	0.53	0.11	0.007	0.032	0.38	12	3.43		Harms (1987)
		0.56	0.17	0.007	0.042	0.35	18	4.05		"
		0.36	0.14	0.007	0.039	0.16	24	3.59		"

Acceptability and digestion of artificial diets together with growth and survival of the larvae of Homarus gammarus (linnaeus).

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## ABSTRACT

While the role of chemoreception in juvenile crustacean feeding behaviour has received considerable attention (Ache, 1982), the chemosensory responses of larval planktonic crustacean stages have been neglected (Yule, 1982).

The acceptability of processed, microparticulate and microencapsulated diets and the possible role for chemoattractants for lobster larvae are investigated. Chance encounter accounts for food capture and the role for low molecular weight chemoattractants appears to be the determination of the edible from the inedible. Dietary conditioning behaviour enables the larvae to discriminate more rapidly between non-digestible and digestible particles. Digestibility and assimilation efficiency studies suggest that lobster larvae have limited enzymatic capabilities, although later stages are more efficient with longer food retention time.

Growth ( $0.114\text{mm day}^{-1}$ ) and survival (80.0%) of lobster larvae, fed natural diets, are significantly better ( $F=14.8$ ,  $p=0.001$  and  $F=54.0$ ,  $P=0.001$  respectively) than when fed artificial diets. Although pre-digested artificial diets gave higher survival (74% and 63%) when compared to microencapsulated diets (37%), there was no significant growth difference between the artificial diets tested.

## INTRODUCTION

The interest expressed in commercial culture of lobsters (Chang and Conklin, 1983) has resulted in the formulation of a variety of purified diets for juvenile stages (Conklin et al., 1983; Kean et al., 1984, 1985), yet little effort has been spent in the development and evaluation of artificial diets for lobster larvae. Although food quality is considered to be a major factor controlling larval recruitment (Scarrett, 1973; Phillips and Sastry, 1980; Sastry, 1983; Castell and Kean, 1986) relatively little is known about the food requirements of larval lobsters (Williams, 1907; Eagles et al., 1984, 1986).

Recent developments and success in the use of microencapsulated diets (Jones et al., 1987; Kurmaly et al., 1989a) have led to accurate determinations of the nutritional requirements of penaeid larvae (Jones et al., 1979b). Similar techniques could be applied to the investigation of nutrition in lobster larvae. In an attempt to satisfy the requirements for particle ingestion, the present work evaluates the acceptability of different dietary and non-dietary items e.g. processed, microparticulate and microencapsulated particles to lobster larvae and investigates the possible role for amino acids as chemoattractants. Growth, survival, diet digestibility, assimilation efficiency and gut evacuation time (GET) of larval lobsters on natural and artificial diets are also determined and their significance discussed.

## MATERIALS AND METHODS.

For each dietary or non-dietary item (Table 1) and attractant (Table 2) tested, different groups of up to fifteen starved larvae were examined individually after an acclimation period of 24 h in 250 ml crystallising dishes. Trials lasted for 1 h, hydrated diets or pieces of filter paper soaked in chemical attractants were presented to the larvae on the end of a needle. The response to each diet tested was categorised as follows; 0 (larvae ignored diet), 1 (larvae accepted and then rejected diet), 2 (larvae ingested diet after handling) and 3 (larvae ingested the diet after active searching). Laboratory manufactured microencapsulated and microparticulate diets were prepared according to Jones et al., (1984) and Kanazawa et al (1977), respectively. Commercial microencapsulated diets were supplied by Frippak Feeds (U.K Ltd). Earlier studies showed that starvation time was an important factor in acceptability trials with lobster larvae. Larvae deprived of food for periods of less than 3h, were more discriminating than those starved for longer than 6 h. Hence, larvae were deprived of food for 3 h before testing in the present study. To assess the response of the larvae to the various attractants, time spent handling the diet was recorded. Diets that were readily accepted were used in further trials to investigate feeding behavior.

To determine growth and survival of larval lobsters on natural and artificial feeds, fifteen larvae were reared on each diet in individual mesh bottom compartments (5x5x5cm), each continuously supplied with filtered (<10  $\mu$ m) seawater at 32.5‰ and 25°C, in a 12 h light:dark regime. Larvae were fed

twice daily after removal of faeces and waste food. Every other day the carapace length (mm) was measured and survival determined. Three trials were completed for each diet tested (see Table 3).

Digestibility trials were conducted with digestive juices and commercial enzyme preparations at various pH. The foregut and hepatopancreas of several larvae were dissected, homogenised and centrifuged in seawater at 0-4°C. The resulting supernatants were mixed with the appropriate buffer solution i.e. Tris maleate buffer for pH 6-8, acetate buffer for pH 3.6-6 and disodium citrate buffer for pH 3.0. Weighed batches of microcapsules were added to the supernatants and mixed in an end over end tumbler at 20°C. Replicates using boiled supernatants served as controls. After 4-12 h, the digest was filtered through a tared Whatman glass fibre filter (G.F.C.: 1 µm pore size) and the weight of material retained was determined. The percentage of diet digested, assumed to be that portion able to enter the hepatopancreatic tubules and be assimilated (Gibson, 1979; Dall and Moriarty, 1983), was estimated from the difference in weight before and after digestion.

Assimilation efficiency on various diets was estimated for several groups of larval or juvenile H.gammarus and Palaemon elegans, using the 'ratio' method (Conover, 1966a,b; Condrey et al., 1972; Moriarty and Barclay, 1981) after pooling samples to give 10-50 mg of faecal material.

Gut evacuation time (GET) was estimated from the minimum and maximum time for total gut clearance for each group of ten individuals, fed to satiation.

## RESULTS

With the exception of the diets bound in agar which are avoided by lobster larvae (Table 1), most diets were handled irrespective of size. Encapsulated mysid flesh incited a positive response when washed thoroughly, omission of the gelatin wash procedure (Kurmaly, 1989) produced noxious microcapsules which were avoided by the larvae. Microencapsulated diets, would only elicit a random search response when flavoured with an attractant. All processed natural diets, however, instigated random and sporadic searches when offered to stages I-III. Stage IV which is benthic and possesses relatively well developed antennules (Hinton and Corey, 1979), showed evidence of distance chemolocation, particularly with frozen rather than freeze-dried food (Table 1).

It was observed that microencapsulated diets were often held onto for long periods by the frontal appendages and at first readily consumed. After 5-15 minutes however, depending on the initial capture rate, once 3-4 particles had been consumed the larvae began to reject these particles with increasing frequency and after 8-10 had been consumed, the rate of acceptance decreased (Figure 1). The number of particles accepted with time (minutes) is given by the following equation:

$$\text{No of particles accepted (Y)} = 1.234 \times (\text{Time})^{0.657}$$

$$\text{S.E of coefficient} = 0.027, r^2 = 87.4\%, N = 88$$

The instantaneous rate of change of particle acceptance with respect to time is given by:

$$dY/dt=0.81(\text{Time}^{-0.34})$$

where Y is the number of particles accepted and time is in minutes. Rejection was not caused by satiation since the larvae continued to accept mysids. A similar pattern of behaviour was noted with chalk and Kaolin for all stages. Such dietary conditioning behaviour was also evident when mussel/albumin particles were offered to stage III. After 1 h particles were not only rejected but avoided.

A selective preference was shown for natural diets, but selectivity appears to decrease as the larvae grow. Thus stage IV lobster larvae were less discriminatory than stage I, particularly to mussel/albumin particles. Artificial diets were more acceptable when flavoured with attractants, hence the role of low molecular weight compounds as taste enhancers was investigated and the results presented in Table 2. The response of the larvae of H.gammarus to pieces of filter paper soaked in various concentrations of amino acids differs markedly from the larvae of P.elegans. Lobster larvae exhibited relatively long handling times for most amino acids, e.g L-glutamic acid, IMP, AMP and trimethylamine whereas L-arginine was the only amino acid to produce a similar response from P.elegans larvae.

In contrast to the results obtained on natural diets, growth and survival of the larvae of H.gammarus on artificial diets was poor (Tables 4a,b & 5a,b); artificial diets did not support growth or survival beyond stage III. While pre-digested diets (diet codes 4 and 5: Table 4a) resulted in significantly higher survival values than microencapsulated diets (Table 4b), with regards to growth there was no

significant difference between artificial diets (Table 5a,b). Observations on lobster larvae fed on artificial diets revealed that the majority of larvae died in mid-moult and were mechanically trapped in their old shells. Whether a dietary ingredient essential for the successful completion of moulting was limiting or missing is not known. Although, growth and survival on artificial diets is low, the results are an improvement on previous attempts to rear lobster larvae on artificial diets. Earlier trials with microparticulate feeds, microencapsulated diets and BML 81S prepared following D'Abramo et al., (1983, 1984, 1985), did not support larvae beyond stage I.

Digestibility trials (Table 6), stress the importance of pre-washing microencapsulated diets with seawater prior to use in feeding experiments. Results show that pepsin is the most effective enzyme, and that trypsin and chymotrypsin are only effective on thin walled crustacean diet (CD) microcapsules, e.g. CD434 (Frippak Feeds U.K Ltd.). The pH range (6-7) over which the digestive juice of larval lobsters is found to be most effective corresponds to the pH reported by Van Weel (1970), Brockerhoff et al., (1973) and Barker and Gibson (1979) for several decapod crustacea.

Assimilation efficiency determined by the 'ratio' method (Table 7) confirms the results of the digestibility trials, indicating that larvae have difficulty in assimilating artificial diets. Later larval stages do however, become increasingly more efficient at digesting artificial diets. Mysids are efficiently assimilated and high values of up to 97% were obtained.

GET (Table 7) increased both with time of starvation and the size of the larvae. The higher assimilation efficiency noted for the older larvae is presumably brought about by the increased residence time within the gut.

## DISCUSSION

The role of chemoreception in juvenile crustacean feeding behaviour has received considerable attention (Grant and Mackie, 1974; Heinen, 1980; Ache, 1982; Carr et al., 1987). It has been shown that antennule chemo-receptors (aesthetascs), can initiate orientation towards food, pereopod chemo-receptors can control capture and conveyance to the mouth where oral receptors can determine ingestion or rejection. Considerably less research has been conducted on chemosensory responses of larval planktonic crustacean stages and has concentrated on omnivorous and herbivorous larvae (Jones et al., 1979; Yule, 1982; Kurmaly et al., 1989a; Clark et al., in press). The present study shows that carnivorous planktonic larval stages do not show orientation behaviour towards a source of food stimulus, but instead rely on chance encounters to capture food items. Particularly, as they possess limited means for locating (Hinton and Corey, 1979) or pursuing feed items through the water column.

Lobster larvae tend to be voracious feeders, grasping and masticating most non-noxious items that come into physical contact with their appendages. Though food capture is largely unselective, ingestion appears dependent on chemical cues and on most occasions only nutritious particles are ingested. On contact with the mouthparts both chemical and textural stimuli become important. Similar observations on the importance of palatability with predatory larvae have been reported by Moller et al., (1979) for Macrobrachium rosenberghii.

Whilst work on fish has shown that betaine significantly reinforces the chemoattractant effect of feeds leading to increased ingestion (Carr et al., 1977; Carr and Chaney, 1976), no similar indication of any one amino acid attractant has been shown for juvenile crustacea (Ache, 1983). Several amino acids, similar to those used in the present study (Table 2), are frequently reported to act as stimulants (Case, 1964; Laverack, 1968; Carr et al., 1983), but no clear hierarchy exists amongst the amino acids tested. Further, only a handful of workers have shown attractants to elicit a response at naturally-occurring concentrations (Ache, 1983). Since chance encounter accounts for food capture the only role for low molecular weight chemoattractants is in the determination of the edible from the inedible (Yule, 1982).

There is in the literature no mention of a dietary conditioning behaviour in predatory planktonic larvae, shown in Figure 1. Since lobster larvae are unable to locate their food items at a distance and rely on chance encounter, it is obviously advantageous to be able to discriminate and not to continually handle low benefit food items, particularly if handling costs contribute significantly to the overall energy budget (Schoener, 1971). Conditioning must play an important role in the feeding strategy of a dietary specialist such as a carnivorous larva; ensuring that it selects not only nutritious items, but also those that are readily digestible. This is of particular importance to organisms which have to maximise their net energy intake from a limited time spent feeding. Whether learning behaviour is controlled by a feedback mechanism related to haemolymph sugar or amino acid content as evidenced in rats (Howard and Yudkin, 1963; Dick

and Felber,1975), or by quality of food influencing enzyme synthesis, postulated by Samain et al.,(1980) is unknown. In either case present results indicate that the lobster will only continue to ingest microencapsulated diets if attractants are regularly alternated.

Given the improvements in larval growth and survival on pre-digested and attractively flavoured diets, and the successful use of similar artificial diets with the larval stages of Penaeus japonicus and P.monodon (Kanazawa et al.,1977; Jones et al.,1979; Kurmaly et al.,1989a) and juvenile lobsters (D'Abramo et al.,1984), it seems unlikely that the poor growth and survival in present experiments is due to nutritional deficiencies within the diets. Frequent handling of the larvae or confined culture space may contribute to the lack of acceptability. Equally the larvae may simply be unable to adequately digest artificial diets.

The absence of any digestive breakdown (Table 6) of CD227, CD288 and CD301 by trypsin and chymotrypsin indicates that thick walled microencapsulated diets, developed to prevent nutrient leaching, may significantly reduce digestibility. The absence of an inhibitory effect on the hydrolysis of CD434 in the presence of soyabean trypsin inhibitor shows that other enzyme systems are present and effective on the walls of encapsulated diets (Table 6). The presence of powerful carbohydrases e.g x amylase in crustacean digestive juice is well documented (Wojtowicz and Brockerhoff, 1972; Van Wormhoudt et al.,1973; Abdel-Rahman et al.,1979; Lee et al., 1980).

Digestibility and assimilation efficiency (Table 6,7) suggest that lobster larvae have limited enzymatic capabilities, although later stages are apparently more efficient probably because GET increases. Changes in enzymatic activity with decapod larval development (Van Wormhoudt et al., 1973, 1980; Laubier-Bonichon et al., 1977; Galgani and Benyamin, 1985), consistently show an increase in amylase and tryptic enzyme activity with age. As lipid is the major energy source for lobster eggs (Pandian, 1967, 1969, 1970; Holland, 1978), it is postulated that perhaps the early stages (stage I), contain a predominance of lipase and esterase enzymes and that systems regulating tryptic and amylase activity are not fully developed (Frank et al., 1975; Anger et al., 1985). The difficulties in rearing fish larvae (Adron et al., 1973; Fluchter, 1980) could well have a similar explanation as low digestive efficiencies have also been shown by Stroband and Dabrowski, (1979). In vitro experiments with carp larvae led Jancarik (1964) to conclude that exogenous enzymes present within natural feeds may support digestive processes in carnivorous fish larvae with poorly developed stomachs. This idea has been developed by Glogowski and Dabrowski (1977a,b), and may also be relevant for lobster larvae.

Additionally, the poorly developed grinding apparatus present in stages I-II (Factor, 1981) and the effects of diet preparation, which can result in the destruction and denaturation of certain nutrients (Forster and Beard, 1973), may also exacerbate digestive efficiency. It is well known that acid halides can covalently bond to C=C bonds, react with sodium salts of fatty acids and perform nucleophilic

substitutions in a variety of reactive groups including -OH and -NH<sub>2</sub>, producing detrimental effects on the nutritional value of dietary components during processing (Kondo, 1979). Hence, although microencapsulated diets have been used successfully with omnivorous larvae (Kurmaly et al., 1989a), their use with lobster larvae in their present form is limited. Success with omnivorous larvae e.g. P.monodon is attributed to a short GET. Whilst P.monodon larvae are able to replenish their guts continually (5-7 times h<sup>-1</sup>: Jones and Kurmaly, 1987), and thus able to satisfy their daily energy needs, even if fed on particles of low digestibility (Kurmaly et al., 1989b). Lobster larvae retain a meal for hours (Table 7) and since this increased feed retention time is not correlated with an improvement in digestibility, lobster larvae are unable to meet their daily energy needs. Subsequently, even if a larva ingests 50mg day<sup>-1</sup> of microencapsulated diet, it will only assimilate 0.55J day<sup>-1</sup> (assuming assimilation is equivalent to the portion of diet reduced to <1µm), which is barely enough for maintenance purposes (Kurmaly and Jones, 1989). Consequently, larval development on diets of low digestibility can only occur when residence time in the gut is short and ingestion virtually continuous.

Although present survival rates are far from encouraging, the possibility of rearing carnivorous larvae on artificial diets remains feasible. Future developments include the testing of diets which incorporate digestive enzymes (Dabrowski and Glogowski, 1977a,b; Maugle et al., 1982) and more easily digested proteins (Kanazawa et al., 1988). The possibility of using cholinergic drugs to influence GET and hence increase the daily throughput of food (Fange and Grove,

1985), will also be investigated.

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Table 1. The Acceptability of different dietary and non-dietary items to the larva of H.gammarus (stage I-IV, pre-starved 3h). Responses are given in percentages. Certain compounds were flavoured in mussel extract.

Compound	Ignored	Response	
		Accept/reject	Accept/ingest Search/ingest
Non-dietary; <sup>3</sup>			
Chalk 1mm <sup>3</sup>	20	80	
Polystyrene 31mm <sup>3</sup>	86*	14	
Kaolin 1mm <sup>3</sup>	100		
Kaolin flavoured 1mm <sup>3</sup>		100	
Gelatin		100	
Dietary;			
i)natural processed diets,			
Mysid frozen			100
Mysid freeze-dried			100
Mussel freeze-dried			100
ii)laboratory manufactured diets,			
a)bound in agar (3%w/w),			
Microparticulate *1	100*		
BML 81S *2	100*		
Egg yolk	100*		
b)bound in wheat gluten (10%w/w),			
Microparticulate *1		100	
BML 81S *2		100	
Egg yolk		100	

**c)microencapsulated**

diets,				
Mysid 500-700µm				
Mysid 500-700µm *3	80*	20	100	
Krill/Albumin			100	
Mussel/Albumin,				
fed to stage I		86		14
" II		54	46	
" III		13	60	27
" IV				100

**Lobster eggs**

d)commercial				100
diets,				
CD 120 500-700µm	13	60	27	
CD 120 500-700µm,				
flavoured (1h)		20	47	33
" (24h)				100

\* avoidance

\*1 Kanazawa et al .,1977.

\*2 D'Abramo et al., 1984.

\*3 omitting gelatin wash.

Table 2. Effect of amino acids on the handling time of lobster larvae. The mean (+S.E.) time spent by the larvae of H.gammarus and Palaemon elegans handling filter paper soaked in various amino acids. 15 larvae were tested for each amino acid .(--- avoidance).

Attractant	Handling time (sec) <u>H.gammarus</u>	Handling time (sec) <u>P.elegans</u>
Seawater	4.32(+0.66)	3.13(+0.44)
L-Glutamic acid		
4x10 <sup>-5</sup> M	23.83(+1.02)	2.71(+0.31)
4x10 <sup>-4</sup> M	7.71(+1.49)	5.54(+0.73)
4x10 <sup>-3</sup> M	8.41(+1.33)	6.72(+1.09)
L-Arginine		
2x10 <sup>-6</sup> M	1.53(+1.25)	3.71(+0.66)
2x10 <sup>-3</sup> M	1.95(+1.44)	11.23(+3.52)
L-Arginine HCl		
2x10 <sup>-4</sup> M	---	6.65(+3.3)
L-Cystein		
2x10 <sup>-4</sup> M	---	1.79(+0.62)

L-Cystein HCL		
2x10 <sup>-4</sup> M	--	2.83(+ <u>-1.12</u> )
Glycine		
2x10 <sup>-6</sup> M	--	2.32(+ <u>1.13</u> )
2x10 <sup>-4</sup> M	--	8.07(+ <u>2.36</u> )
L-Tyrosine		
2x10 <sup>-6</sup> M	1.28(+ <u>0.55</u> )	8.45(+ <u>1.03</u> )
2x10 <sup>-2</sup> M	1.85(+ <u>0.71</u> )	7.23(+ <u>1.74</u> )
Betaine		
2x10 <sup>-2</sup> M	7.21(+ <u>1.3</u> )	7.24(+ <u>0.7</u> )
(IMP) Inosine 5		
monophosphoric acid		
2x10 <sup>-2</sup> M	28.51(+ <u>1.4</u> )	6.30(+ <u>1.38</u> )
(AMP) Adenosine 5		
monophosphate		
2x10 <sup>-4</sup> M	18.36(+ <u>0.72</u> )	8.22(+ <u>0.74</u> )

Taurine		
$2 \times 10^{-2} \text{M}$	--	--
Leucine		
$2 \times 10^{-2} \text{M}$	--	$1.74(\pm 0.34)$
Trimethylamine		
$2 \times 10^{-2} \text{M}$	$13.12(\pm 1.35)$	$2.73(\pm 0.65)$

Table 3. Dietary treatments tested on lobster larval growth-survival trials.

Diet code	Diet
1	Lobster larva (frozen).
2	Mysids (frozen).
3	CD 434 (255-500 $\mu$ m) pre-soaked for 1h in mussel extract, fed to stage II.
4	9.6g pre-digested BML 81S (1:250 w/w Pancreatin pH 7.8), 0.33g mazola oil, 0.2g wheat gluten, 2.5gDW mysids, made to pH 6.5 .
5	9.6g pre-digested Kanazawa diet (1977) (1:250 w/w Pancreatin pH 7.8), 2gDW mysids, 0.2g wheat gluten, made to pH 6.5 .
6	CD 434 (255-500 $\mu$ m) pre-soaked on alternate days for 2h in a) 1:1,IMP and Trimethylamine $2 \times 10^{-3}$ M, b)1:1, AMP and L-glutamic acid $2 \times 10^{-3}$ M and c) mussel extract.

Diets were stored at -20° C and thawed before use.



Table 4b. Scheffe's multiple range test for the 5% level. A \* denotes pairs of groups significantly different at the 5% level. Diet coded as above.

DIET	6	3	4	5	1	2
CD 434 'flavoured'	6					
Prefed mysid/CD 434	3	*				
Pre-digested BML 81S	4	*				
Pre-digested Kanazawa diet	5	*	*			
Lobster larva	1	*	*	*		
Mysids	2	*	*	*	*	

Table 5a. Mean growth (carapace length:mm) of the larva of H.gammarus after 16 days fed on selected diets. The mean growth of three trials is given. Standard error and the number of samples measured are in parentheses.

		Dietary treatment					
		Natural diets		Artificial diets			
		Mysids	Lobster larva	Prefed mysids/CD 434	Pre-digested Kanazawa diet	Pre-digested BML 81S	CD434 'flavoured'
		1	2	3	4	5	6
Growth (mm)		4.47 (0.113) (63)	3.85 (0.085) (120)	3.55 (0.053) (49)	3.43 (0.055) (64)	3.27 (0.051) (55)	3.16 (0.065) (40)
Stage of 50%		IV	IV	III	III	III	III
Growth*1 rate mm day-1		0.114	0.076	0.056	0.049	0.039	0.032

Table 5 contd.

\*1 assuming carapace length is 2.646mm at T=0.

Oneway anova: F=14.80; P=0.001, significant at the 1% level, lobster larval growth is effected by diet type.

Table 5b. Scheffe's multiple range test at the 5% level. A \* denotes pairs of groups significantly different at the 5% level.

DIET	6	4	5	3	1	2
CD 434 'flavoured'	6					
Pre-digested BML 81S	4					
Pre-digested Kanazawa diet	5					
Prefed mysids/CD 434	3					
Lobster larva	1	*	*	*	*	
Mysids	2	*	*	*	*	*

Table 6. Effect of crustacean digestive juice (d.j), and commercial enzymes on microencapsulated diets, in vitro. Digestibility is recorded as the mean percentage of four trials of diet lost when filtered through a Whatman GFC filter paper, Standard error is given in parentheses.

Enzyme source	Enzyme ratio (ml:mg)	substrate (250-500µm)	Diet code	pH	Digestion time (h)	Percentage breakage(<1µm)
<b>H.gammarus:</b>						
d.j of 150 stage 1 larvae.	1:50		CD 227	3.8*	12	--
d.j of 100 stage 1 larvae.	1:50		CD 227	8.1	12	--
d.j of 200 stage 1 larvae.	1:25		CD 434	8.1	6	32.00(0.91)
d.j of 100 stage 1 larvae.	1:25		CD 434	6-7	6	51.75(1.18)
d.j of 100 stage 1 larvae.	1:25		CD 434*1	6-7	6	25.50(1.50)
d.j of 100 stage 1 larvae*1.	1:25		CD 434	6-7	6	33.25(1.97)
d.j of juvenile.	1:50		CD 227	3.8*	12	--
d.j of juvenile.	1:50		CD 227	8.1	12	--
d.j of juvenile*1	1:50		CD 434	6-7	4	58.50(1.33)
<b>C.maenus:</b>						
d.j of juvenile.	1:50		CD 227	3.8*	12	--
d.j of juvenile.	1:25		CD 227	8.1	12	--
d.j of juvenile.	1:25		CD 434	6-7	5	49.50(1.33)
d.j of juvenile*1.	1:25		CD 434	6-7	5	52.25(1.32)

P.elegans:						
d.j of juvenile.	1:50	CD 227	3.8*	12	--	
d.j of juvenile.	1:50	CD 434	6-7	6	57.75(2.65)	
Commercial enzymes*2:						
Pepsin solution	1:250	CD 227	1.8	6	69.00(2.48)	
"	1:250	CD 288	1.8	6	72.00(1.23)	
"	1:250	CD 301	1.8	6	68.75(0.75)	
Protease solution	1:200	CD 227	7.8	12	32.75(1.88)	
"	1:200	CD 288	7.8	12	30.00(1.87)	
"	1:200	CD 301	7.8	12	50.75(3.09)	
Chymotrypsin solution	1:200	CD 227	8.1	12	--	
"	1:200	CD 288	8.1	12	--	
"	1:200	CD 301	8.1	12	--	
Trypsin solution	1:200	CD 434	6-8	6	29.00(1.87)	
"	1:200	CD 227	8.1	6	30.00(2.58)	
"	1:200	CD 288	8.1	6	34.75(1.49)	
"	1:200	CD 301	8.1	6	--	
"	1:200	CD 434	6-8	6	55.25(1.49)	
Lipase solution	1:250	CD 434	7.7	6	--	
x Amylase solution	1:250	CD 434	7.7	6	44.25(1.70)	
p Amylase solution	1:250	CD 434	7.7	6	--	
p Glucosidase solution	1:250	CD 434	5.0	6	--	
Diastase solution	1:250	CD 434	7.0	6	45.25(2.29)	
Cellulase solution	1:250	CD 434	4.0	6	--	

\* microcapsules not washed in seawater.

\*1 soyabean trypsin inhibitor.

\*2 Enzyme substrate ratio mg/mg.

Table 7. The influence of diet and starvation on the gut evacuation time and assimilation efficiency of the larval or juvenile stages of H.gammarus and P.elegans. Ten individuals were tested on each dietary treatment; minimum and maximum values are given.

Stage	Diet	Satiation time (min)	Time for first faecal pellet (h)	GET 100% Assimilation (h)	efficiency (%) 'ratio'
H.gammarus:					
I	CD 434	5-20	2-3	10-13	16-48
I	Mysids	5-10	1-3	10-12	87-96
I	BML 81S	5-25	3-4	10-15	13-25
I	BML 81S*1	5-20	2-4	10-13	20-35
IV	CD 434	5-20	2-4	16-20	23-46
IV*2	CD 434	5-20	10-12	42-46	24-55
IV	Mysids	5-20	2-3	10-12	88-97
V	CD 434	5-15	5-7	33-36	22-51
V*2	CD 434	5-20	5-8	40-45	20-61
X	CD 434	5-25	5-7	33-38	29-66
X*2	CD 434	5-25	11-13	42-47	27-71
P.elegans:					
Small*3	CD 434	5-20	6-7	18-24	42-68
Large*4	CD 434	5-20	6-8	22-28	35-72
*1	pre-digested				
*2	pre-starved for 48 h				
*3	carapace length = 0.738 cm				
*4	carapace length = 0.928 cm				

Figure 1. Cumulative number of microencapsulated particles CD434 (255-355 um), accepted by lobster larvae (stage 1) with time (minutes). The natural logarithmic regression equation is :

Number of particles handled (ingested, O and rejected, \*) with time.

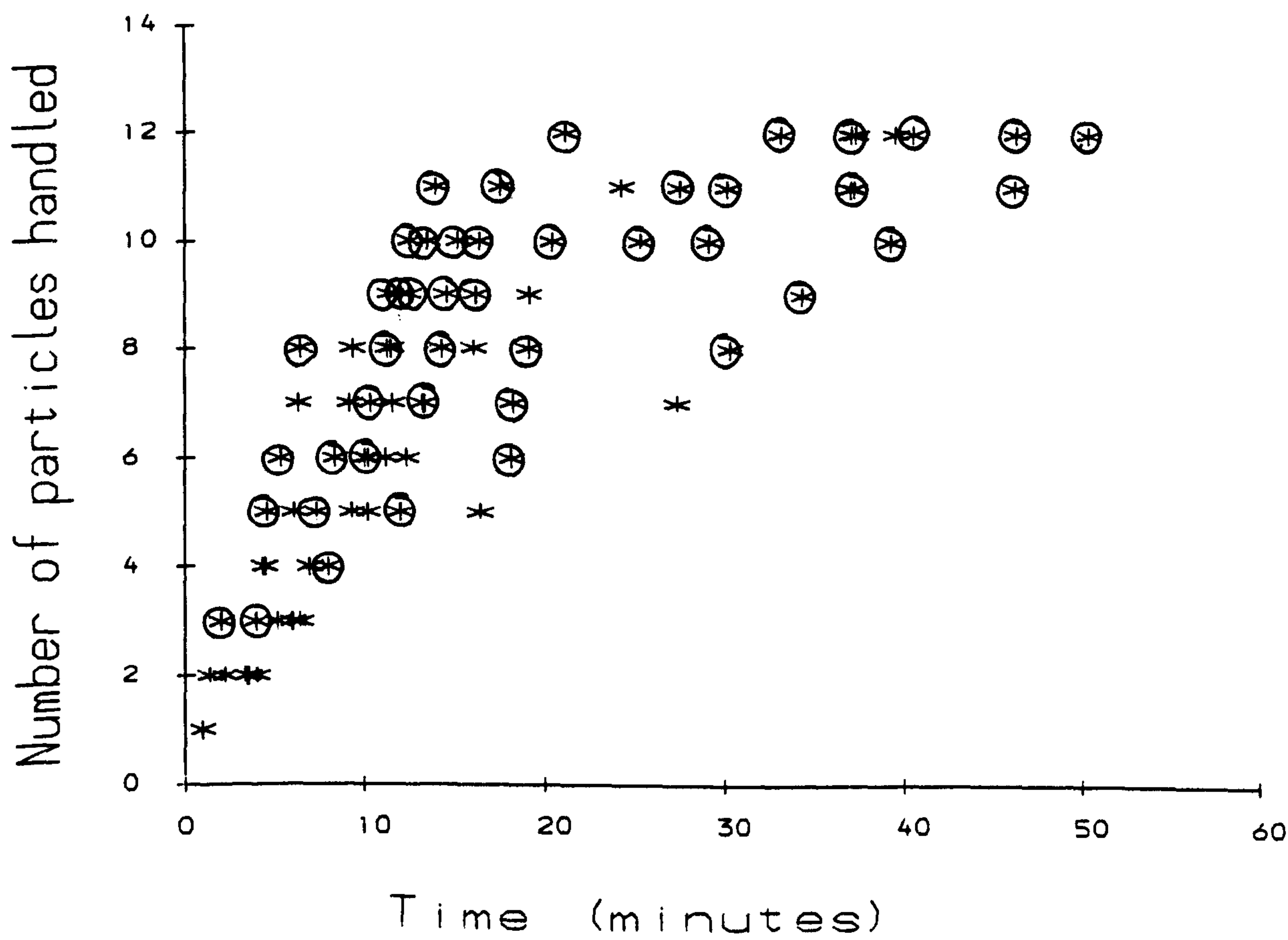
Number of particles handled =  $1.234 \times (\text{time})^{0.657}$  .

S.E = 0.027,  $r^2 = 87.4\%$ , N = 88.

Instantaneous rate of change of particles accepted with respect to time is;

$dy/dt = 0.81 (\text{time})^{-0.34}$ ,

Figure 1



**General discussion.**

Sastry (1983) demonstrates the difficulties in separating cause from effect in the evolution of life history strategies of organisms when our knowledge of selective pressures, resource availability, environmental stability, competition and predation is still essentially speculative. Nevertheless, life history and reproductive strategies can be used to rationalise differences between organisms (MacArthur and Wilson, 1967; Parry, 1981), since they appear to be selective compromises with respect to the combination of biotic and abiotic factors in a particular environment (Christiansen and Fenchel, 1979; Sastry, 1983). Life history patterns and reproductive strategies are adapted to provide maximal survival of the progeny and development to reproductive maturity.

The two species used in this investigation, P.monodon and H.gammarus, have quite different life strategies P.monodon can be viewed as an r-selected species and H.gammarus as a k-selected species (Odum and Pinkerton, 1955; MacArthur and Wilson, 1967; Pianka, 1976; Stearns, 1976, 1977). P.monodon corresponds to an opportunistic, early successional organism of small adult body size, maturing after only 6 months (Primavera, 1978) through rapid growth (Liao et al., 1983), high fecundity (0.1- 7 million eggs: Muthu and Laxminarayana, 1977), showing semelparity, oviparity, absence of parental care and great dispersive capabilities by releasing eggs hence, planktotrophic larvae into the water column (Allen, 1972; Omori, 1974). H.gammarus corresponds to a late successional organism of large adult body size (Passano, 1960; Phillips et al., 1980) which occupies more permanent or predictable habitats where competitive ability to pursue food,

resistance to predation (Cooper and Uzmann, 1980), greater longevity (Herrnkind, 1980), higher energetic investment per offspring (Hepper and Gough, 1978; Sassaki et al., 1986; Attard and Hudon, 1987) ovoviparity and iteroparity (Aiken and Waddy, 1980) are at a premium. Generally, the greater investment per offspring shown by H.gammarus results in smaller numbers ( $5-17 \times 10^3$ ) of eggs (Grassle and Grassle, 1977; Hepper and Gough, 1978), longer (2 years) ovarian cycle and egg incubation periods (>11 months) (Bradford, 1978; Aiken and Waddy, 1980; Todd and Doyle, 1981) and larger eggs, 2-4 mm as opposed 0.2 to 0.4mm diameter, than P.monodon (Hepper and Gough, 1978).

The role of the pelagic larval stage in the life histories of benthic species has been discussed by Thorson (1950), Crisp (1974a,b) and Scheltema (1975). Storage strategies (large or small eggs) are products of selection, and as such, the difference in egg size probably arises from the trade off between propagule size and expectation of survival (Crisp, 1987). Propagules tend to be small where predation pressure is high and large where resource competition is severe (Thorson, 1950; Pianka, 1970, 1976). Egg size has important consequences for the size of larvae at hatching (McLaren, 1965, 1966; Wear, 1974; Crisp, 1987). Lobster eggs contain sufficient quantities of yolk to allow a more developed and larger hatchling which is able to feed carnivorously (Chapter 7 and 8). The small eggs of P.monodon contain sufficient yolk for only a limited embryonic developmental phase (Primavera, 1985). P.monodon eggs hatch to give a non-feeding nauplius which during development transfers through several trophic levels (Chapter 3, 5 and 6).

Both species spend between 12-18 days in the plankton or in culture (Chapter 3,5 and 7). The lobster larvae moult 4 times before becoming benthic whilst the P.monodon larvae moult 12 times, between nauplius 1 and benthic postlarva 1. Considering the difference between developmental stage at hatch, short time span in the plankton, predation pressure, subsequent need to become benthic, and difference in maintenance and feeding costs (Kiorboe et al.,1982; Logan and Epifanio, 1979), it is not surprising that P.monodon larvae have a higher G/M ratio and net growth efficiency (K2) than H.gammarus larvae (Chapter 3 and 7). The former develops from a non-feeding planktonic nauplius to an omnivorous benthic feeder with a hundred fold increase in dry weight. In contrast the latter increases in dry weight by a factor of 12, in 12-18 days.

Work on crustacean larval digestive physiology has demonstrated that the digestive enzyme mix, activity and substrate utilised correspondingly changes as the larvae develops (Frank et al.,1975; Sulkin et al.,1975; Capuzzo and Lancaster, 1979; Laubier-Bonichon et al.,1977; Samain et al.,1985; Galgani and Benyamin, 1985). Quite likely, if larval development is associated with a rapid progression through trophic levels, it may be uneconomical to construct an efficient digestive system specialising at any one trophic level (Chapter 4). The P.monodon larva has compensated for a low assimilation efficiency (10-24%) by having a GET that is determined by food availability. Since prolonged digestion time will not necessarily result in corresponding increase in digestive efficiency, optimal energy gain is best achieved if the larva increases its food throughput (Kurmaly et

al.,1989a). This strategy promotes optimal foraging from an environment where resources consist largely of detrital particles of low energetic and nutritious value (Pomeroy, 1980), particularly when the larvae have no ability to distinguish edible from inedible particles. Kiorboe et al.,(1985a,b) state that such a strategy confers low maintenance requirements and is well suited to unstable coastal environments where sediment and detrital material are continually resuspended. It is postulated that such a life style pre-adapts this prawn for growth and survival on artificial diets which are not readily digestible (Chapter 2).

Lobster eggs hatch to produce an organism which is immediately carnivorous (Van Olst et al.,1980) and in this respect is relatively similar to the juvenile. Since there is no drastic change in feeding habits between early and later stages, the initial larval stage is a dietary specialist (Williams, 1907), with an efficient and advanced digestive system evolved to feed on readily digestible live animal foods (Chapter 8). Such prey items are not only digestible but themselves contain enzymes that may contribute to the digestive process (Lauff and Hofer,1984). High values for assimilation efficiency (75-85%) are achieved by efficient digestive enzymes and extended gut residence times, where the rate of digestion determines ingestion and defaecation. Furthermore, the gut is unable to cope with particles of low digestibility and conditioning behaviour plays an important part in reducing the time and energy wasted on non-digestible particles (Chapter 8).

Consequently organisms normally showing high assimilation efficiencies might be expected to be less adaptable to fluctuating nutritive conditions, unless compensatory digestive mechanisms can adjust the rates of ingestion, digestion and assimilation. Differences in gastro-evacuation rates and their subsequent effects on absorption efficiency have also been shown for herbivorous and carnivorous fishes (Fange and Grove, 1979; Hofer, 1982). Indeed, surveys have shown that herbivorous species like carp and mullet are the most successfully cultured. Whether this means that the culture of carnivorous organisms will be limited to live or processed natural feeds depends on further feeding trials with microencapsulated diets, incorporating readily digestible proteins, digestive enzymes or cholinergic drugs (Chapter 8).

The present study identifies the major energetic differences between larvae occupying different trophic levels and attempts to explain the contrasting growth and survival of P.monodon and H.gammarus achieved on artificial diets. If these differences are general to the meroplankton care must be taken in the organisms selected for culture on microencapsulated diets or detrital material. Organisms with satiable omnivorous or predominantly carnivorous larvae, high assimilation efficiencies and slow evacuation rates controlled by the digestion rate are unlikely to be suitable candidates.

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## APPENDIX 1.

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## APPENDIX 2.

# **PENAEID SHRIMP HATCHERY TRIALS**

## **USING MICROENCAPSULATED DIETS**

by

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## ABSTRACT

A microencapsulated particulate feed, developed to replace live foods conventionally used in penaeid larval culture, has been used in preliminary trials during 1984-5 in commercial larval hatcheries. This feed is composed of a balanced crustacean diet, microencapsulated within a biodegradable capsule wall designed to protect the internal diet from bacterial degradation while allowing leaching of food attractants.

This encapsulated feed may be used to replace part or all of the conventional live foods used in penaeid larval culture. If the feed is maintained in suspension, larval survival of P.monodon, P.vannamei and P.stylirostris may exceed that obtained on live foods. Simple suspension devices, capsule sizes and feeding rates are described.

Excess feeding was not necessarily harmful, but adjustment of larval stocking densities can maximise feeding rate efficiency to a level where  $1 \times 10^6$  postlarvae are produced for less than 2.6 kg of encapsulated feed.

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## APPENDIX 3.

1

## PENAEID LARVAL REARING ON MICROENCAPSULATED FEEDS

by

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### ABSTRACT

Research is targeted at the total replacement of all live feeds currently employed in the larval culture of penaeid shrimps by microencapsulated artificial feeds. Cross-linked protein walled microcapsules have been developed in a range of sizes from 5-500  $\mu$ m diameter, containing balanced crustacean dietary formulations. This paper presents results of studies using these feeds under both hatchery and laboratory conditions. Results of recent trials demonstrate, for Penaeus monodon, enhanced growth and survival (80-100%) for mysis and post-larval stages when fed encapsulated diets as a replacement for live feeds of rotifers and Artemia. However, only minor improvements in growth and survival were obtained for Z1-Z3 stages over the algal fed controls. From parallel laboratory rearing studies it is concluded that particle size is a major factor limiting the performance of encapsulated feeds for the early algal feeding larval stages. As soon as capsule size is reduced below 10  $\mu$ m similar patterns of feeding behaviour, particle ingestion, digestion and faecal pellet production to algal fed controls are observed. The superior performances of the latest encapsulated feeds for the predatory mysis and post-larval shrimp stages appear to be related to further improvements in formulation and increased retention of water soluble elements such as vitamins within the microcapsules.

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