

## Cellular Ultra Structural Damage by Heavy Metal Cadmium Toxicity \*Hussain . A. Sheriff

### الخلاصة:

تمت دراسة التأثيرات السمية لمعدن الكاديوم الثقيل على البنية الداخلة خلوية المنظورة تحت المجهر الالكتروني وذلك بفحص خلايا الغلاصم التنفسية للحروريات من رتبة ذبذبات مايس للمياه العذبة ( L ) ( Cloeon dipterum ). وقد اظهرت النتائج وجود التلف والتخريب في هذه الخلايا والذي يتناسب تصاعديا مع التراكيز التجريبية .  
بعد تعريض العينات التجريبية لفترة 36 ساعة ( وهي الفترة الاختبارية ) اللازمة لقتل 50% من العينات . لقد اظهرت صور المجهر الالكتروني كافة التشوهات الحاصلة في العضيات الخلوية مثل (الغلاف النووي, المايوتوكندريا, الشبكة الاندوبلازمية والتنظيف الطبقي للصفحة الغلصمية) فضلاً عن زيادة تكوين المشتملات الدهنية والفسح والحوصلات السايوبلازمية, بينما لم يتأثر جهاز كولجي على الاطلاق وذلك في التراكيز العالية ( 1PPM, 0.5PPM ) .

ولقد اظهرت الصور المتحصل عليها حقيقة التخريب والتلف الحاصل في العضيات الامر الذي يفسر الاسباب الحقيقية التي تقف وراء موت الخلايا المتأثرة بالتلوث وبالتالي يمكن على ذلك تفسير اسباب الوفيات في الحيوانات والانسان عند التعرض الى التسمم بالمعادن الثقيلة . كما تم تناول الاسباب الفارماكولوجية الناشئة من التعرض للمعادن الثقيلة ودورها في اتلاف انزيمات السلفادرايل والتي ستؤدي الى الخفض الشديد في معدلات تنفس الخلية , مما يضيف عاملاً ثانوياً يعجل من موت الخلية اضافة الى التلف والتخريب السالف الذكر في العضيات

### ABSTRACT

The toxic effect of heavy metal ( Cadmium ) was investigated using a species of freshwater aquatic insect - nymph of Ephemeroptera , Cloeon dipterum ( L.) . This heavy metal was found to have a significant effect on the fine structure of the gills in proportion to the concentration of Cadmium used ( 0.1 ppm, 0.5 ppm and 1 ppm ) . The results were supported by ultra structural observation on different types of the gills cell organelles ( gill lamella , nucleus , mitochondria , endoplasmic reticulum and other parts of the cell ) . Transmission electron microscopy has revealed the disruption and the damage for the normal cellular organization of the nymph gill cells when the animal exposures for 36 hours in the pollutant media . Most of the freshwater pollution – scientists thought that this kind of aquatic insects can used as biological monitors of heavy metal pollution . The aim of the present study is to investigate the poisoning effects of this heavy metal on the mortality caused by ultra structural damage which is standing behind the death of the animal and human been .

# **INTRODUCTION**

The concern over Cadmium pollution has been largely related to its effects on humans, as a result of consumption of this metal in high concentrations bioaccumulated in the organisms used as food. This has been a matter of great concern to food and drug agencies in many countries. Invertebrates are considered excellent indicator organisms because of capability in concentrating metals, among other pollutants. With the growing interest in aquaculture, heavy metals have an important practical aspect. Scientists say that although there has been a great deal of interest in and considerable research on metal pollution during the last few years as a result of human poisoning, there is still much to be learned about the effects of metals on the aquatic organisms, particularly at the sub lethal level.

If we are to be able to manage our renewable resources properly in the face of growing technology and industrial production, such new learning must be acquired soon.

Understanding of the effects of water quality changes on aquatic organisms will require knowledge of organism population and community levels of organization and it will require synthesis of this knowledge, so that biological interaction may be comprehended. Biochemists, physiologists, pharmacologists, behaviorists and ecologists interested in the effects of water quality changes on aquatic organisms will need to be enlisted in the pursuit of this knowledge.(8)

many studies have been made to show that a given species is associated with water quality, the association are usually restricted to very limited geographical areas.

Furthermore, the predictability of the results is not high enough to allow the associations to be used as a biological test for water pollution. For example, there was a report indicating that clouds of mayflies emerge from the water of the upper Mississippi River during the summer. These clouds of mayflies comprise a nuisance for individuals living along the shores of the river and for traffic on the river. The hordes of mayflies which emerge from the river are associated with zones of the river which are clean and relatively unpolluted. Along zones of the river which are polluted, there is virtually no mayflies nuisance. two areas of the Mississippi river are specially noted in that report for failure to produce significant numbers of mayflies. Both of these areas are Known to be badly polluted by heavy metals waste materials. other studies have indicated that the mayfly is a pollution sensitive organism. it has been suggested that the presence of these pollution sensitive forms indicates that the river is more river – like than sewer – like in a given area.

The object of the present study has been to contribute to knowledge of the role of heavy metal Cadmium in important fresh water aquatic insects ( Ephemeroptera ) by showing the effects of this metal on the cell ultra structural damages which are clear in the cell organelles of the animal tested. this metal was chosen since it shows a varying degrees of pharmacological toxicity in many kinds of organisms including human been (2).

The studied nymph of *C. dipterum* has seven pairs of gills situated laterally along the abdomen. The first six pairs each consists of two lamellae. These gills move with a metachronal rhythm which causes

water to flow between them and along the abdomen so they are play an important role on oxygen consumption. Since 1973 Eastham(4), reported that the well developed tracheal system, which passes through the basal part of the gill lamella, so they gave a leaf – shape to each gill ( figure 1- A ). The ultra structural anatomy of each gill cell shows two multilayer's of cuticle ( outer and inner ), inside we can see the different types of organelles such as nucleus, mitochondria, Golgi complex whereas in the middle center,

the round section of trachea wall surrounded by the lipid inclusion spots are so clear .( figure 1- B ) .

## MATERIAL AND METHODS

Samples of freshwater aquatic insects nymphs ( Cloion dipterum ) were collected from its natural habitat using a net sweeping over submerged plants . After collection , the nymphs were transferred to the laboratory and kept in glass tanks containing habitat water . All experimental nymphs were fed with submerged plants from the same habitat on alternate days during this holding period lasting for one week . After this period , standard – sized individuals ( 3 – 6 mm) were acclimated to the required temperatures (  $20\text{C}0 \pm 1$  ) for at least 48 hours before use . All the experimental animals were starved for 12 hours before use . Aqueous stock solutions of 1.000 part per million (ppm) of the metal ion of hydrated Cadmium chloride ( ANALAR ) ( $\text{Cd cl}_2 \cdot 2\frac{1}{2} \text{H}_2\text{o}$ ), was prepared by dissolving 2.0316 gr in one liter of glass- distilled water, this stock solution was renewed every two days . In order to obtain concentrations of 0.1 , 0.5 and 1 ppm , 1 ml of part per thousand was made up to 1 liter by the addition of glass - distilled water to obtain a stock solution of 1 ppm . 100 ml and 500 ml of 1 ppm solution were made up to 1 liter by the

addition of glass – distilled water to obtain a stock solution of either 0.1 or 0.5 ppm respectively . All the glassware used in the experiments was acid washed with dilute nitric acid . All the stock solutions were kept at  $20\text{C}0 \pm 1$  before use , and freshly made up every two days .For electron microscopy ,life specimens were placed in 5% glutaraldehyde in buffered sodium cacodylate with sucrose added , and there gills dissected out . These tissues was then placed in fresh 5% glutaraldehyde solution at pH 7.4 for one hour , washed in several changes of buffered sodium cacodylate with sucrose added at  $0 -4\text{C}0$  , followed by post – fixation in 1% osmium tetroxide buffered with sodium cocodylate with sucrose added for one hour and then washed for one hour in ice – cold glass – distilled water . After dehydration in graded cold acetones the tissue was embedded in TAAB embedding resin . Suction with gold or silver interference colours were obtained using a Cambridge Instrument Huxley Mark1 ultra microtome and were mounted on coated copper grids . They were then double stained in 30% Uranyl acetate in methanol (30 minutes ) , followed by saturated lead citrate (10 minutes ) , and viewed in a Corinth AEI electron microscope .(8) .

## DISCUSSION

The period of exposure for the all three experimental concentrations was 36 hours and this was chosen because the lethal time 50% value for these nymphs was 36 hours in the higher concentration solution of 1 ppm Cadmium ions . After exposure to 0.1 ppm Cadmium for 36 hours there was a significant decrease in the number of mitochondria compared with that in control specimens,( $P > 0.01$ ) .

The spherical shaped mitochondria ( m ) ,( figure 3-1 ), become more numerous than the elongate shaped ones( figure 2-3 ) . There is a significant reduction, however, the number of cristae compared with the control specimens (  $p > 0.05$  ) . ( figure 3 -1),( figure 2-3) in 0.1 ppm exposure it was clear that no damage happened for each of rough endoplasmic reticulum (rer) and nuclear membrane (nm) whereas the cuticle starts to loose its distinct zones . The Lipids inclusion (Li) and vesicles (v) become more evident in this concentrations . The cuticle of insects and other crustacea have similarities (5) . Both consist primarily of two layers, the exocuticle , not containing chitin and the endocuticle,a laminated chitin – protein complex . The epicuticle in both possesses very thin surface

layer of different composition . The outer thin epicuticle , always rich in lipid substances , may be termed the «lipoid epicuticle» and the inner , basically composed of protein as the « protein epicuticle » . (6). Cadmium ions may have a harmful effect on protein content of the cuticle . Papathanassiou and king ( 1983 )(7), reported that Cadmium ions have been shown to have an effect on the fine structure of the gill cells of two species of mayflies , and it may affect several functions of the gill cells such as enzymic and ATPase activity , absorption and transportation of salts , active ion uptake and protein synthesis .

Lipid inclusion aggregated surrounding the trachea showed an increase in tissues contaminated with high concentrations ( 0.5 ppm and 1 ppm Cadmium ) . Which may indicate that Cadmium ions affect lipid metabolic functions(fig.4-2,5), (fig.5-4) .Since 1969 Baker (1),reported that high and medium levels of copper poisoning in the winter flounder resulted in fatty metamorphosis in the liver , and there was fat in the cells around the central vein .

In 0.5 ppm Cadmium had an effect on the gill lamelle by reducing the number of organelles present in the epithelial cells . As in specimens from 0.1 ppm , there were fewer mitochondria than in control specimens as it mentioned before and most mitochondria were spherical in shape and had fewer cristae than in control specimens . Some of the mitochondria had a swollen appearance (fig. 4-3,4) and others continued degenerating cristae or cristae free areas ( fig. 4 – 1,3 ,4 ) . The aggregation of lipid inclusions surrounding the trachea was more pronounced in specimens from either control or 0.1ppm .

In 1ppm Cadmium for 36 hours there were fewer organelles present in the epithelial cells than from either control , 0.1ppm or 0.5ppm ( fig. 5, fig. 6 ) . The epithelial cell nucleus was still large in relation to the cell but its outer membrane was disrupted in places ( fig. 5 – 1 ) .

There were fewer mitochondria present than on control specimens ( $p > 0.02$ ) but was no significant reduction in the number of mitochondria compared with specimens from 0.5ppm (  $p < 0.1$  ) while there was a significant reduction in the number of mitochondria compared with those present in specimens subjected to 0.1ppm (  $p > 0.001$  ) .

1ppm Cadmium chloride had a deleterious effect on all cell organelles , the inner cuticle had lost its distinct zones ( fig.5–2 ),most of the mitochondria were completely degenerate . The lipid inclusion were more aggregated around the trachea ( fig.5–4 ) . The numerous vesicles (v) had appeared with large spaces between the cell organelles (fig.6). The invaginations of apical plasma membrane , although still closely applied to the cuticle , were smaller than in either control specimens or those exposed to 0.1ppm or 0.5ppm Cadmium chloride (fig.5-1).

There was no effect on Golgi complex in all concentrations .

In general conclusion Cadmium ions specially in high concentrations 0.5ppm and 1ppm have been shown to produce deteriorative effects on gill structure and the functioning of cell organelles of the animal . Since 1961 Basso et al (2), explained the more dangerous effects standing behind the cell death by heavy metal poisoning from pharmacological point of view , they reported that within the cells , small amounts of soluble substances containing free sulphhydryl groups exert and inhibitory effect on the activity of sulphhydryl enzymes which control the rate of respiration . the removal of free sulphhydryl groups by a combination with some heavy metals to an acceleration of respiration and at high concentration the metal become attached to the enzyme molecules them selves and inhibition of oxygen uptake follows . This fact may be stand behind the cell death as an accessory reason which is added to the organelles damage happening when heavy metal poisoning take place .

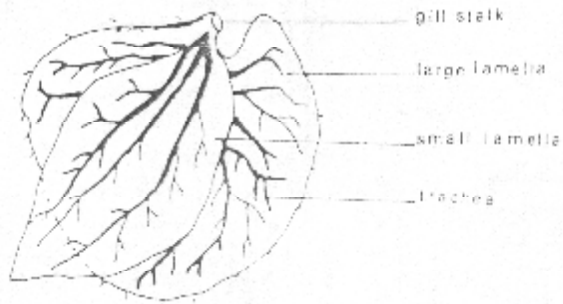
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Fig.1-A. Diagram of gill Cloeon dipterm .

Fig. 1-B. Diagrammatic representation of a gill epithelial cell.

A.



B.

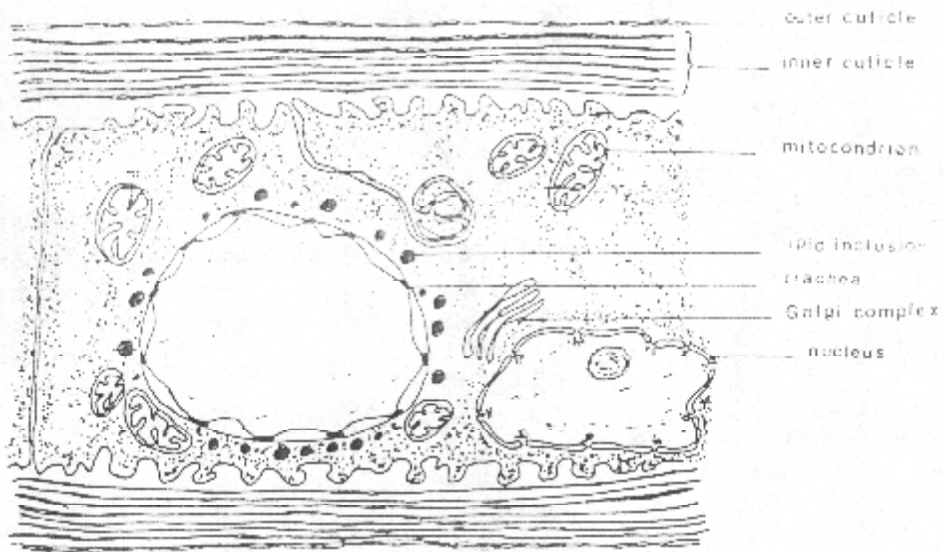


Fig. 2-1. Electron micrograph showing section of the gill lamella, showing the cuticle (cu) , the apical plasma membrane (ap) and the rough endoplasmic reticulum (rer) .  
Scale bar = 0.5  $\mu\text{m}$  .

Fig. 2-2. Electron micrograph showing section of the gill lamella ,showing the distinct layers of the cuticle (cu) , the apical plasma membrane (ap)  
Scale bar = 0.25  $\mu\text{m}$ .

Fig. 2-3. Electron micrograph of section through the gill lamella central cell showing the mitochondria (m) and the rough endoplasmic reticulum (rer)  
Scale bar = 0.5  $\mu\text{m}$  .

Fig. 2-4. Electron micrograph showing part of the trachea (t) and the mitochondria (m) surrounded by the rough endoplasmic reticulum(rer)  
Scale bar = 0.5  $\mu\text{m}$  .



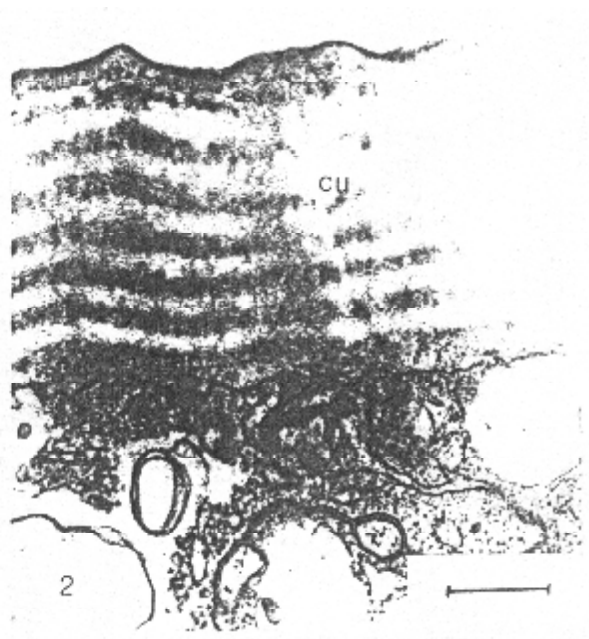
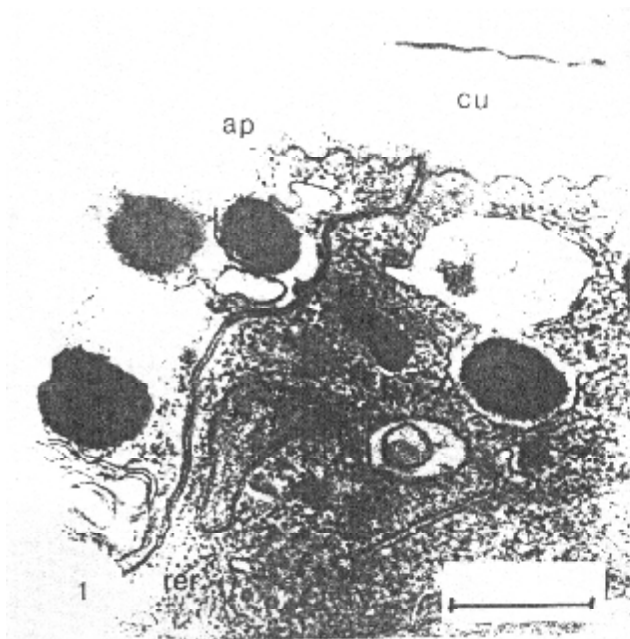


Fig. 3-1. Electron micrograph of section through the gill lamella central cell showing the rough endoplasmic reticulum(rer) , after exposure to 0.1 ppm Cadmium chloride . Note the spherical shape of the mitochondria (m) and the presence of the vesicles (v) . Scale bar = 0.5  $\mu$ m .

Fig. 3-2. Electron micrograph showing the cuticle (cu) and the mitochondria (m) after exposure to 0.1 ppm Cadmium chloride . Scale bar = 0.5  $\mu$ m .

Fig. 3-3. Electron micrograph showing the nucleus (N) and the nuclear membrane (nm) , after exposure to 0.1 ppm Cadmium chloride . Scale bar = 0.5  $\mu$ m .

Fig. 3-4. Electron micrograph showing part of the trachea (t) and the aggregation of lipids inclusion(li) Scale bar = 0.5  $\mu$ m .

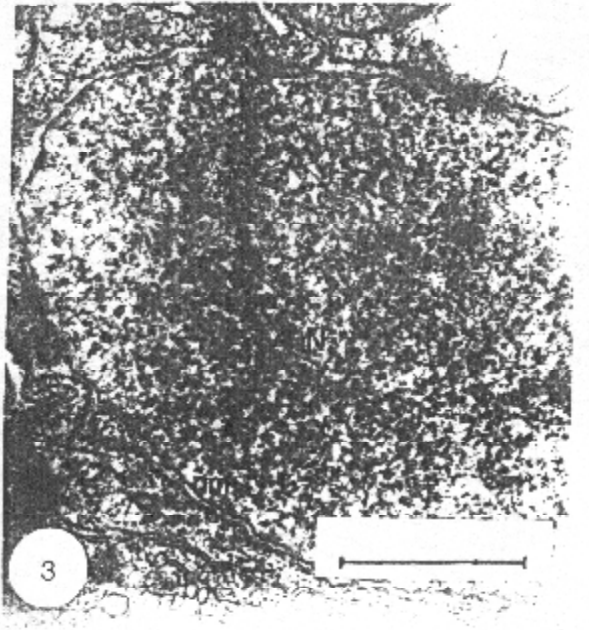
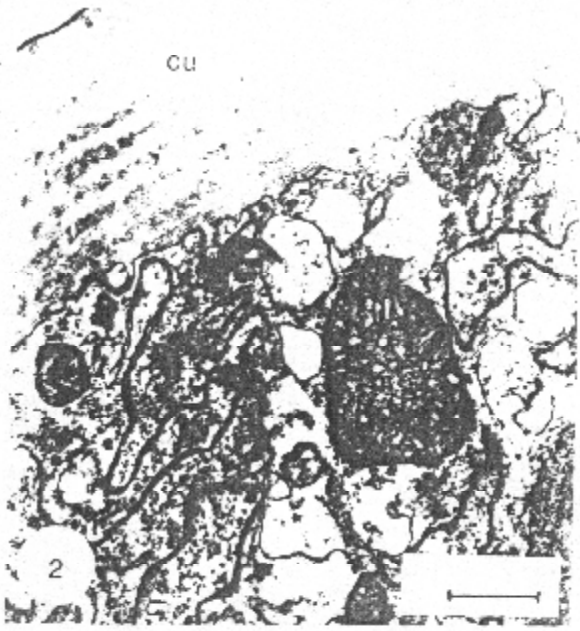


Fig. 4-1. Electron micrograph of section through the gill lamella central cell . after exposure to 0.5 ppm Cadmium chloride . Note the swollen appearance of the mitochondria (m)  
Scale bar = 0.5  $\mu\text{m}$  .

Fig. 4-2. Electron micrograph of the whole section of the gill after exposure to 0.5 ppm Cadmium chloride . Note the disappearance of the cuticle layers (cu) and trachea (t) , the increase of the aggregation of lipids inclusion(li). ( arrow). Scale bar = 0.25  $\mu\text{m}$  .

Fig.4-3. Electron micrograph of section through the gill lamella central cell . after exposure to 0.5 ppm Cadmium chloride .Note the degeneration of some of the cristae ( arrow heads).  
Scale bar = 0.5  $\mu\text{m}$

Fig.4-4. Electron micrograph of section through the gill lamella central cell . after exposure to 0.5 ppm Cadmium chloride .Note the cristae free areas in the mitochondria ( arrow heads).  
Scale bar = 0.5  $\mu\text{m}$

Fig.4-5. Electron micrograph showing part of the trachea (t) and the aggregation of lipids inclusion(li) after exposure to 0.5 ppm Cadmium chloride .  
Scale bar = 0.5  $\mu\text{m}$ .

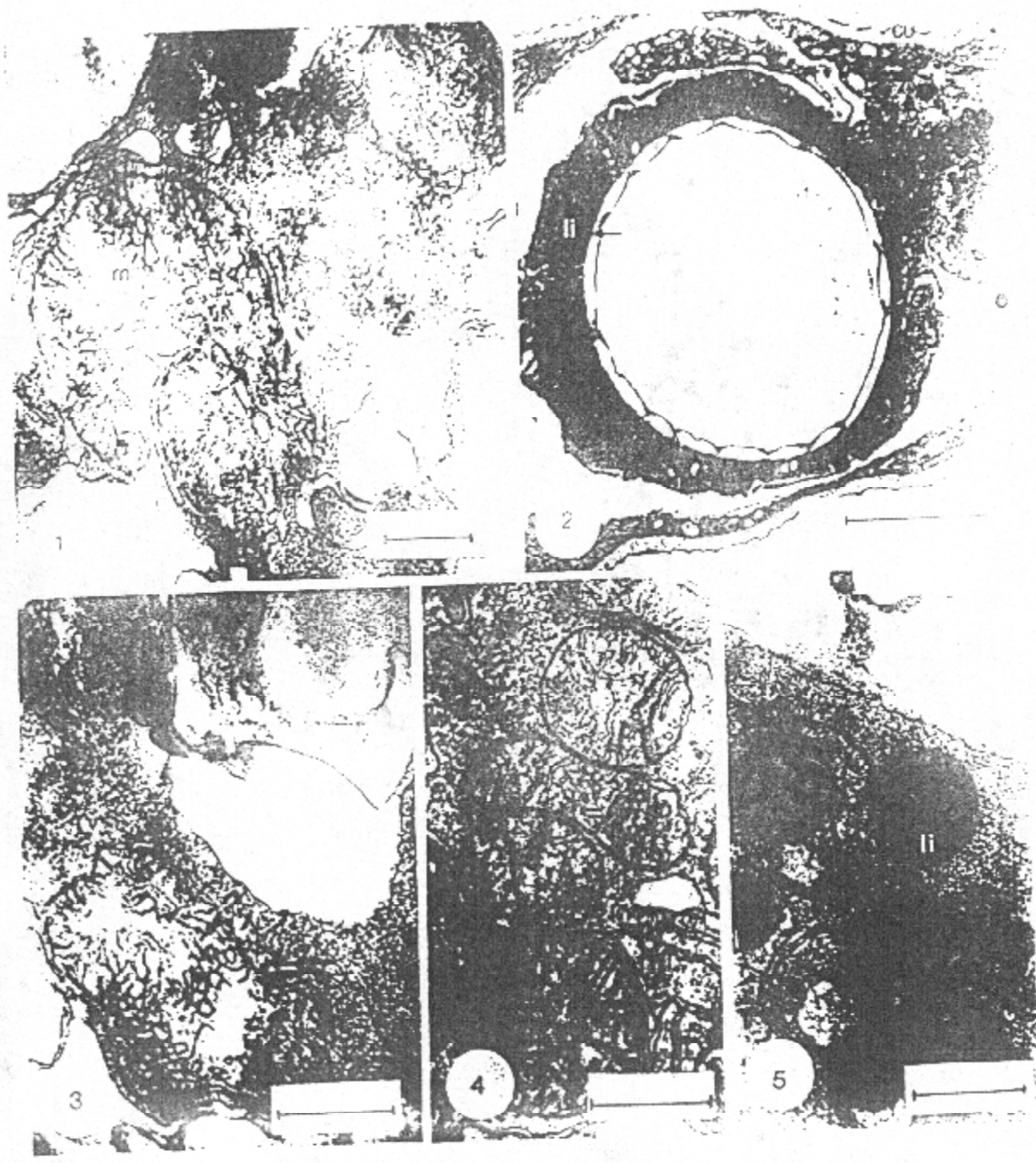


Fig.5-1. Electron micrograph showing the nucleus (N) after exposure to 1 ppm Cadmium chloride . Note the Golgi complex (Gc ) , the disappearance of the inner cuticular zones (cu) and the outer membrane of the nuclear envelope disrupted in places (arrowheads ). Scale bar = 1  $\mu$ m.

Fig.5-2. Electron micrograph showing the degeneration of all cell organells , after exposure to 1ppm Cadmium chloride . Scale bar = 0.25  $\mu$ m.

Fig.5-3. Electron micrograph showing the degeneration of mitochondria (m) , after exposure to 1ppm Cadmium chloride . Scale bar = 0.5  $\mu$ m.

Fig.5-4. Electron micrograph showing the increase of aggregation of the lipids (arrow) . Scale bar = 0.25  $\mu$ m.

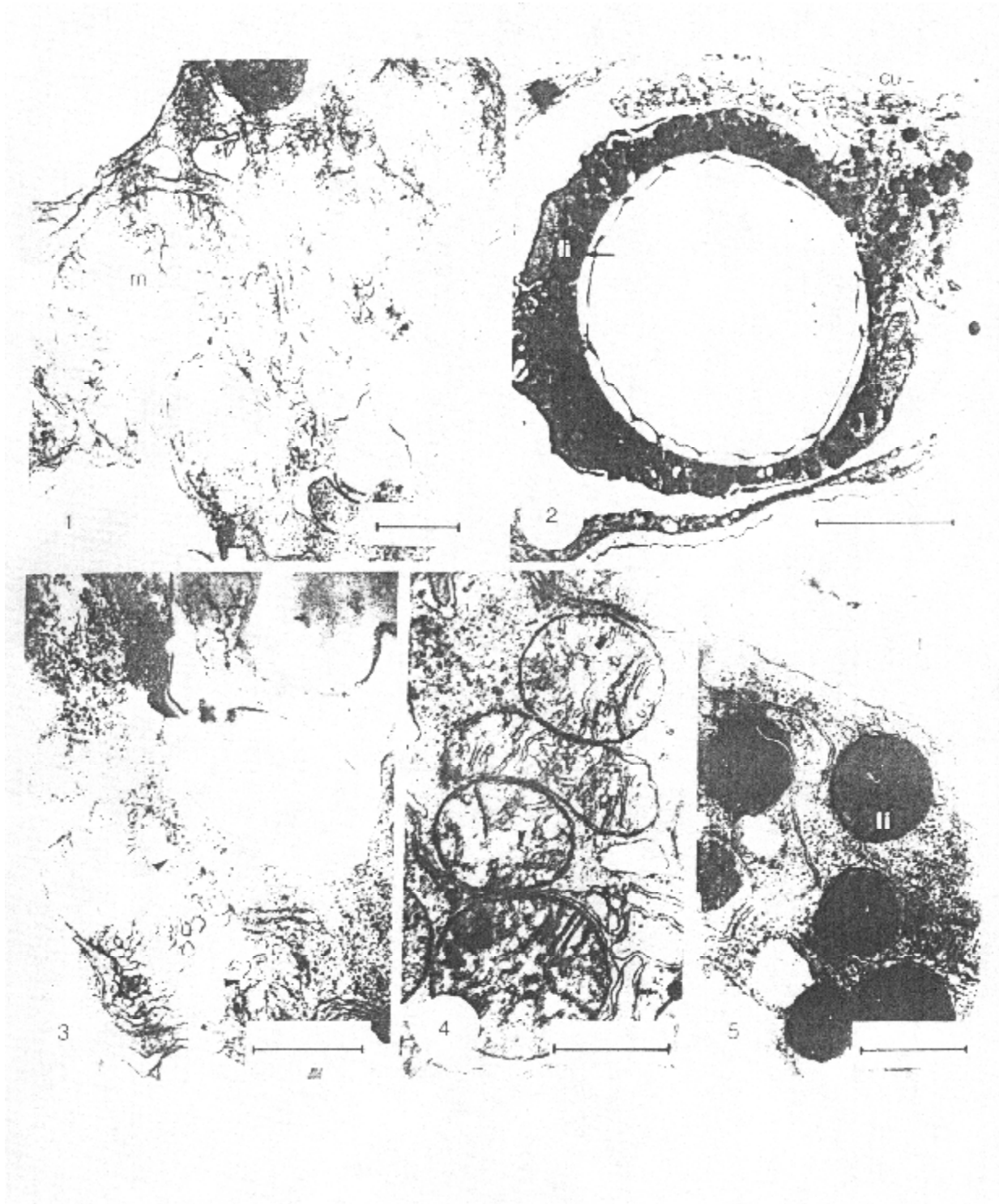


Fig.6-1. Electron micrograph showing the degeneration of all cell organelles, after exposure to 1ppm Cadmium chloride. Note the nucleus (N), the appearance of the numerous vesicles (v) and the large spaces between the cell organelles (s).  
Scale bar = 0.5  $\mu\text{m}$ .

Fig.6-2. Electron micrograph showing the appearance of the numerous vesicles (v) and the degeneration of the mitochondria (m).  
Scale bar = 0.5  $\mu\text{m}$ .

Fig.6-3,4. Electron micrograph showing the degeneration of all cell organelles, after exposure to 1ppm Cadmium chloride. Note the large vesicles (v) and the large spaces between the cell organelles (s).  
Scale bars = 0.5, 0.25  $\mu\text{m}$ .



