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镉对长江华溪蟹精子发生的影响*

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摘 要 分别于1999年3～5月和10～12月, 利用透射电镜方法, 按1.5 μg/g体重的剂量体腔注射Cd^{2+}于长江华溪蟹(*Sinopotamon yangtsekiense*)体内, 在10 min至24 h之间的若干时间段取材, 观察精子发生过程中不同发育阶段生殖细胞的变化。结果表明: 镉注射后0.5 h, 各级生精细胞就开始出现超微结构的变化, 主要表现为线粒体肿胀与空泡化, 高尔基体变形, 内质网扩张和膜结构损伤等。其中线粒体损伤出现最早, 是最易受损伤的一个敏感细胞器。

关键词 长江华溪蟹 精子发生 镉中毒 超微结构 甲壳纲

随着现代工业的发展, 有毒重金属的污染日益严重。一般认为, 镉在体内呈长期性蓄积对动物生殖功能具有潜在性危害(Favler, 1982; 郭永灿等, 1989)。目前, 研究者多以哺乳动物为材料, 研究重金属对其生殖腺的影响(徐晨等, 1997; 李维信等, 1988); 在水生生物则多以鱼类为代表, 研究重金属对其肾脏和生殖腺的影响(郭永灿等, 1989)。而镉对甲壳动物十足类生殖细胞超微结构的影响, 迄今为止, 未见报道。作者在对长江华溪蟹进行了精子发生一系列研究的基础上(王兰等, 1996, 1997, 1999, 2000), 就镉对其精子发生的影响及其作用机制做了进一步的研究与探讨。

1 材料和方法

1.1 实验动物

实验用长江华溪蟹购自太原市花鸟鱼虫批发市场, 放室内50×30×32 cm水族缸中饲养, 期间投喂鱼饲料、蚯蚓或土豆, 3～4天换水一次, 水质为暴晒2～3天的自来水。

1.2 实验毒剂

氯化镉(AR级)用生理盐水配制成母液。

1.3 实验方法

取15只中等大小且健康活泼的成熟雄性个体, 分别称体重, 平均体重12 g。按1.5 μg/g体重的剂量, 体腔注射Cd^{2+}于中等大小的蟹体中(李维信等, 1988), 然后将染毒蟹放入20×20×22 cm水族缸中暂养。在Cd^{2+}处理后的各个时间段(10 min, 0.5 h, 1 h, 2 h, 4 h和24 h)内, 活体解剖雄蟹, 迅速取出精巢, 先放入22.5%戊二醛中固定2 h, 再放入1%锇酸中固定2 h。丙酮梯度脱水, 618环氧树脂包埋, 瑞典LKB-5型超薄切片机切片, 切片指示厚度为500-700, 醋酸双氧铀～柠檬酸铅双重染色, 日本JEM-100CX透射电镜观察并拍照。10～12月重复一次。

2 结 果

实验结果表明, Cd^{2+}对长江华溪蟹精子发生各期生精细胞均有不同程度的影响。

2.1 精原细胞

镉注射10 min后, 未见精原细胞有明显的超微结构异常。0.5 h至2 h, 观察到个别精原细胞有细微的变化; 4 h后, 可见部分精原细胞的细胞质膜部分破裂, 细胞核变形, 线粒体绝大部分空泡化。随着处理时间的延长, 细胞器形态的变化不断加重(图版Ⅰ: 1)。

2.2 初级精母细胞

镉注射10 min后, 未见初级精母细胞有明显的超微结构变化。0.5 h至1 h, 初级精母细胞形态正常, 质膜和核膜结构完整, 高尔基体基本正常; 个别线粒体嵴有消失现象; 细胞质中出现大量内质网小泡, 多呈棒状, 紧靠核外膜分布(图版Ⅰ: 2)。2 h后, 细胞核多数变形, 核外膜膨胀,

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向外突起呈波浪形；核仁增多，3～5 个不等，有的核仁移位至核边缘；线粒体空泡化增多，部分线粒体缩小；核糖体增多（图版 Ⅰ：3～5）；高尔基体略有变形（图版 Ⅰ：6）；滑面内质网增多，分泌小泡变得不规则呈扩张状。4 h 后，大部分初级精母细胞发生变化，表现为细胞膜破裂；细胞核变形，呈多边形、长椭圆形、镰刀形等，核染色质凝聚成块状；线粒体嵴消失，空泡化程度加重。24 h 后，细胞核严重变形，染色质有所减少；线粒体不但空泡化，而且有变形；高尔基体也有变形。另外，在细胞质中出现较多溶酶体。

2.3 次级精母细胞

镉注射后 10 min，次级精母细胞无明显的超微结构改变。0.5 h 后，细胞结构基本完整，形态较规则。细胞质中线粒体部分变形，但高尔基体无明显变化；1 h 后，细胞膜部分解体，细胞核开始变形，核外膜有轻微膨胀（图版 Ⅰ：7），线粒体有的外膜破裂，嵴不完整（图版 Ⅰ：8），内质网产生许多空泡（图版 Ⅱ：9），高尔基体、溶酶体基本正常（图版 Ⅱ：10）。2 h 后，细胞核变形为柱状，线粒体嵴部分消失，有的内部呈絮状；滑面内质网扩张（图版 Ⅱ：11），出现不规则空泡（图版 Ⅱ：12）；核糖体脱落；高尔基体变化不大；溶酶体正常。4 h 后，大部分次级精母细胞质膜与核膜破裂，核染色质游离，细胞质内有许多空泡（图版 Ⅱ：13）。24 h 后，细胞核变形呈花边状；线粒体内部呈絮状或空泡化；内质网严重扩张、空泡化且不规则（图版 Ⅱ：14）；高尔基体基本保持正常。

2.4 精细胞和精子

镉注射后 10 min，精细胞和精子无明显超微结构变化。0.5 h 至 2 h，有的精子细胞膜局部凸起。4 h 后，个别精子的细胞膜严重外凸，成伪足样，顶体变形似马鞍状（图版 Ⅱ：15）。24 h 后，精细胞外膜部分破裂；精子细胞膜变得不规则，顶体有的呈马鞍状，有的拉长成椭圆形。线粒体分布在顶体中（图版 Ⅱ：16）。

3 讨 论

3.1 镉对长江华溪蟹精子发生的影响

长江华溪蟹精子发生的研究已有较详细的报道（王兰，1996，1997，1999，2000）。经电镜观察，镉注射后各级生精细胞超微结构均发生了一系列的形态变化。镉注射 2 h 后，精原细胞出现超微结构的改变，且随着注射时间的延长病变逐渐加重，如细胞质膜破裂，细胞核变形，线粒体空泡化等。镉注射后，初级精母细胞细胞器出现超微结构变化的顺序为：线粒体、内质网（0.5 h）、细胞核、核外膜（2 h）、细胞膜、高尔基体、溶酶体（24h）。次级精母细胞细胞器出现超微结构变化的顺序为：线粒体（0.5 h）、细胞膜、核膜、内质网（1 h）。镉注射后 0.5 h，精子细胞膜出现变化；4 h 后，顶体变形，线粒体移位。

从以上结果可以看出，除了精原细胞，各级生精细胞在镉注射后 0.5 h，均出现了不同程度的形态学改变，主要表现为膜结构的损伤，其中，线粒体损伤出现最早，说明线粒体是细胞内最易受损伤的一个敏感细胞器，可显示细胞受损伤的程度。

3.2 镉中毒原理

人类镉慢性中毒可引起 Ca^{2+} 代谢异常，导致骨骼软化。钙和钙调素二者结合在一起，协调细胞内各种依赖于钙的过程，并可激活细胞内的主要酶类，如蛋白激酶、磷酸脂酶、核苷酸环化酶、离子通道蛋白和依赖于 Ca^{2+} 的 ATP 酶等，从而在维持细胞形态、有丝分裂等过程中发挥重要作用。由于镉与钙具有相近的离子半径和相似的化学性质，二者具有拮抗作用。镉主要作用于细胞膜，与膜蛋白巯基结合，阻止 Ca^{2+} 的跨膜内流，最终导致细胞内游离钙的缺少，使依赖于钙的 ATP 酶、钙调素不能被激活，从而影响细胞的正常代谢与 DNA 的模板作用，同时也可引起微管和纺锤体解聚，干扰细胞的有丝分裂过程（张笑一，1997；Ei-Ichiro，1995）。此外，也有人认为，镉主要损伤需锌等微量元素激活的酶系统，它与巯基、羧基及含氮配基结合，其亲和力比锌大，因此体内一些含锌酶中的锌被镉取代而丧失其固有功能（李维信，1988）。尚有实验证实，镉对肝细胞内线粒体的氧化磷酸化过程，对于肾素、淀粉酶、还原酶、木瓜蛋白酶、过氧化物酶、转移酶、羧化酶等活性均有抑制作用。由于镉可以抑制超氧化物歧化酶的活性，加快细胞脂质过氧化的速率，导致脂质过氧化物的堆积，进而损伤膜结构，改变膜的通透性，最终引起细胞器的分解（刘瑞明，1990）。本实验观察到，体腔注射镉后，各级生精细胞超微结构均出现了损伤，表现为线粒体肿胀、空泡化，核外膜破裂，高尔基体变形、囊泡扩大，内质网扩张且空泡化，细胞膜膨胀及破裂等，这些变化都属于膜损伤后导致的细胞肿胀、溶解性退变过程。提示各级生精细胞超微结构改变系镉的直接损伤而引起。

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外 文 摘 要 (Abstract)

EFFECTS OF CADMIUM ON SPERMATOGENESIS IN FRESHWATER CRAB (SINOPOTAMON YANGTSEKIENSE) *

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Reproduction of Crustaceana is a complex regulated process which can be affected in several ways by heavy metals and other pollutants. For the first time the effect of cadmium on the male germ cell of the freshwater crab (*Sinopotamon yangtsekiense*) was studied by the means of body cavity injection (Cd^{2+} 1.5 μg/g boby weight) in this paper. The germ cell at the different developmental stages in the treated and control individuals had been observed by transmission electron microscopy (TEM) in order to determine whether cadmium might have a morphological effect on them during spermatogenesis. The same experiment had been done twice in the period of March to May and October to December in 1999.

In the experiment, male crabs were brought from aquatic product market in Taiyuan and raised in laboratory for more than a week before treatment. The mature male crabs in middle size were chosen and injected Cd^{2+} into the body cavity. The treated and control animals were dissected in different times (10 min, 0.5 h, 1 h, 2 h, 4 h, 24 h). The results showed that all kinds of observed cells had been changed ultrastructurally after 30 minutes of cadmium treatment except for spermagone, and that the longer of cadmium treatment was, the more seriously the cells had been changed in morphology.

Spermagone had ultrastructural changes after two hours of cadmium treatment; and their cell membrane had been broken partly and nuclear membrane distorted and most mitochondria vacuolated after four hours of treatment. In primary spermatocyte and secondary spermatocyte, ultrastructural changes had been observed after 30 minutes of Cd^{2+} treatment, and changes of organelle were distinct after one hour of Cd^{2+} treatment. The damaged order of organelle of spermatocyte was: mitochondria and endoplasmic reticula firstly (30 min), nuclear membrane and nucleoli secondly (2 h), cell membrane and Golgi complex and lysosome lastly (24 h). In secondary spermatocyte order, mitochondria had been damaged firstly (0.5 h) and cell membrane and nuclear membrane and endoplasmic reticula secondly (1 h). Injected cadmium 30 minutes later, the cell membrane of spermoblast had been damaged and acrosome had been distorted and mitochondria had been shifted 4 hours later, i. e. injecting cadmium into the animals could cause all organoid of all sorts of male germ cells damaged shortly. Among five kinds of male germ cell, spermagone was the latest one to be damaged by cadmium. Golgi complex was the least to be damaged by cadmium, its ultrastructure had been observed normally in secondary spermatocyte II and had been damaged slightly in primary spermatocyte I after 24 hours of treatment. Cell membrane and mitochondria were affected earlier than other organelle. The changes of cell membrane and mitochondria were including cell membrance swelled seriously (eg. spermoblast) and disintegrated partly, mitochondria distorted and vacluolated and broken, some mitochondria contracted and turned smaller than normal ones, some

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mitochondria cristae disappeared. At first nucleus had been distorted and nuclear membrane had been expanded slightly , chromatin of primary spermatocyte had been condensated to cluster and chromatin of secondary spermatocyte had been turned free 4 hours later. Ribosome and nucleoli had been increased to three or five , and some of the latter had been shifted around nuclear membrane 2 hours later. Rough endoplasmica reticula (RER) had been vacuolated largely and turned into smooth endoplasmica reticula (SER) by disassemblying ribosome from RER , which led to the increase of SER and ribosome. There were more lysosome and vacuolus in cytoplasmic matrix and other organella had been damaged seriously twenty$^{3}\!\!\diagup$four hours later.

The results showed that male germ cells of the experimented animals could be damaged by injecting cadmium into their body cavity. The organella with membrane structure were easily damaged , such as mitochondria , nucleus , endoplasmica reticula and cell membrane. Among them mitochondria was damaged earliest , which suggested that mitochondria was a sensitive organelle to cadmium and could show damage degree led to by cadmium.

Key words　Freshwater crab (*Sinopotamon yangtsekiense*) , Spermatogenesis , Cadmium , Ultrastructure , Crustacea

图版说明 (Explanation of Plates)

图 版 Ⅰ (Plate Ⅰ)

1 Cd^{2+}处理 4 h 后的精原细胞，线粒体空泡化 [Vacuolation of mitochondria in spermatogonium after 4 h of Cd^{2+} treatment] ×20 000

2 Cd^{2+}处理 30 min 后的初级精母细胞，线粒体嵴消失，内质网小泡呈棒状 (↖) [Disappearance of mitochondrial cristaes and baculiform vacuolation of endoplasmic reticulum in primary spermatocyte after 30 min of Cd^{2+} treatment] ×8 000

3 Cd^{2+}处理 2 h 后的初级精母细胞，细胞核变形，核仁增多 (↖) [Distorted nucleus and increasing nucleoli in primary spermatocyte after 2 h of Cd^{2+} treatment] ×10 000

4 Cd^{2+}处理 2 h 后的初级精母细胞，线粒体空泡化严重 (↖) [Occurrence of severe vacuolation of mitochondria in primary spermatocyte after 2 h of Cd^{2+} treatment] ×8 000

5 Cd^{2+}处理 2 h 后的初级精母细胞，核糖体增多 (↖) [Increasing ribosomes in primary spermatocyte after 2 h of Cd^{2+} treatment] ×14 000

6 Cd^{2+}处理 2 h 后的初级精母细胞，高尔基体略有变形 (↖) [Slightly distorted Golgi complex in primary spermatocyte after 2 h of Cd^{2+} treatment] ×8 000

7 Cd^{2+}处理 1 h 后的次级精母细胞，细胞核变形 [Distorted nucleus in secondary spermatocyte 1 h after Cd^{2+} treatment] ×6 000

8 Cd^{2+}处理 1 h 后的次级精母细胞，线粒体嵴不完整 (↖) [Incomplete mitochondrial cristaes in secondary spermatocyte after 1 h Cd^{2+} treatment] ×15 000

Cr: 线粒体嵴 (cristae) M: 线粒体 (mitochondria) N: 细胞核 (nucleus) R: 核糖体 (ribosome)

图 版 Ⅱ (Plate Ⅱ)

9 Cd^{2+}处理 1 h 后的次级精母细胞，内质网空泡化 (↖) [Vacluolated endoplasmic reticulum in secondary spermatocyte 1 h after Cd^{2+} treatment] ×14 000

10 Cd^{2+}处理 1 h 后的次级精母细胞，示高尔基体和溶酶体 [Golgi complex and lysozyme in secondary spermatocyte 1 h after Cd^{2+} treatment] ×16 000

11 Cd^{2+}处理 2 h 后的次级精母细胞，内质网扩张 (↖) [Expanded endoplasmic reticulum in secondary spermatocyte 2 h after Cd^{2+} treatment] ×8 000

12 Cd^{2+}处理 2 h 后的次级精母细胞，示不规则空泡 (↖) [Irregular vacuole in secondary spermatocyte 2 h after Cd^{2+} treatment] ×10 000

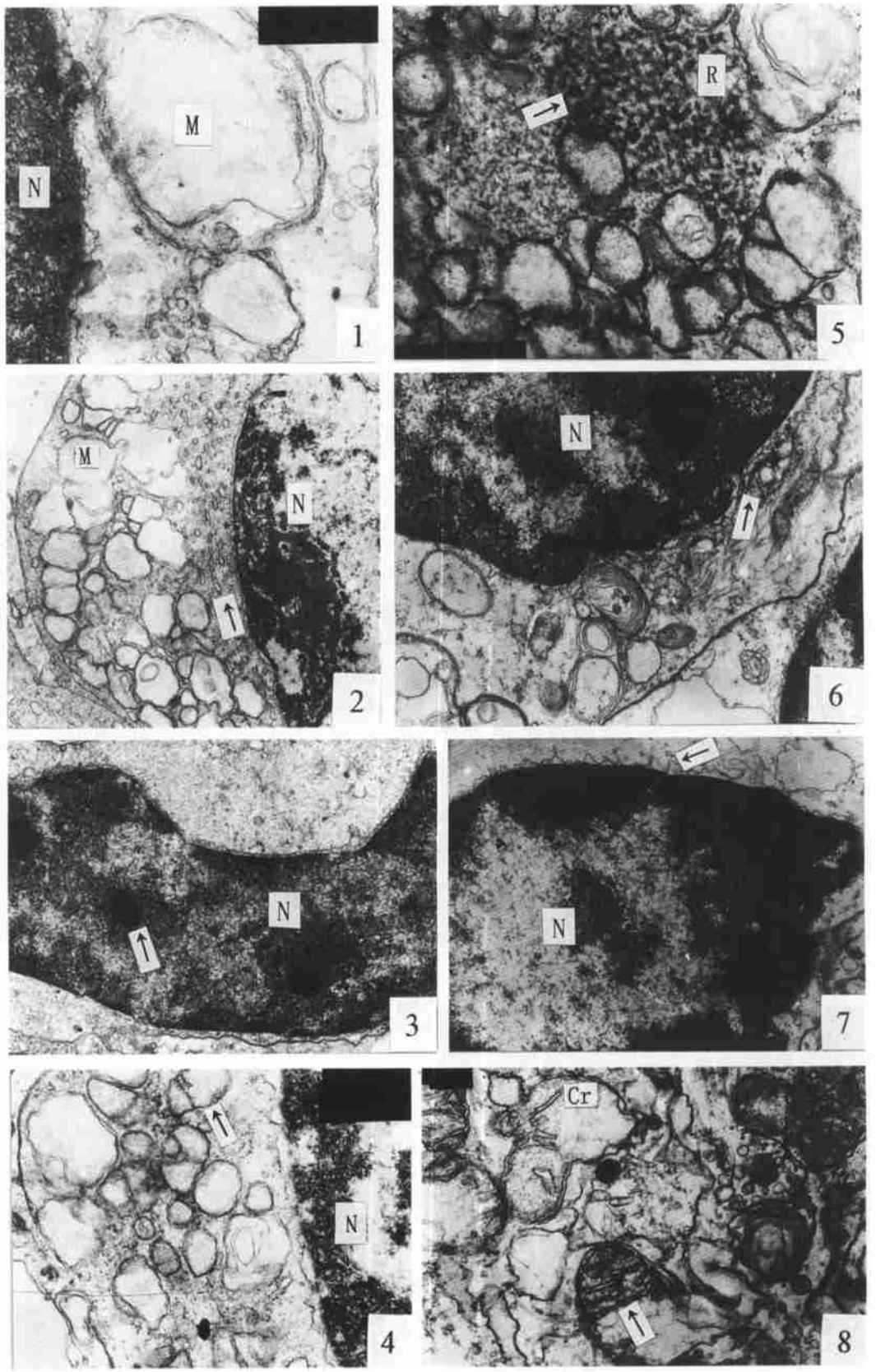
13 Cd^{2+}处理 4 h 后的次级精母细胞，核膜破裂 (↖) [Disorganized neclear membrane in secondary spermatocyte 4 h after Cd^{2+} treatment] ×10 000

14 Cd^{2+}处理 24 h 后的次级精母细胞，核变形 (↖) [Distorted nucleus in secondary spermatocyte 24 h after Cd^{2+} treatment] ×8 000

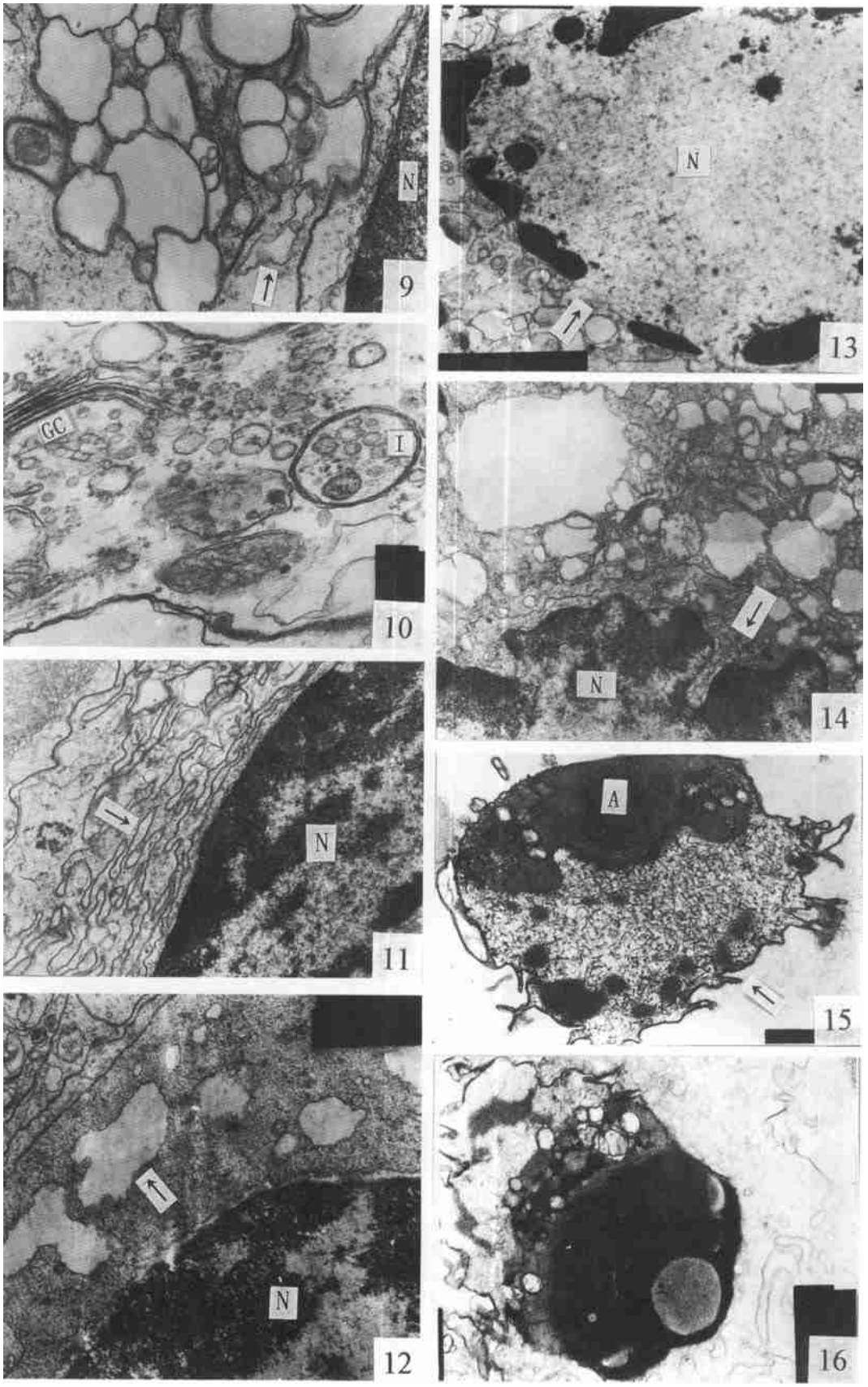
15 Cd^{2+}处理 4 h 后的精子，顶体变形，细胞膜外凸 (↖) [Abnormal acrosome and cytoplasm is heaved outwardly in sperm 4 h after Cd^{2+} treatment] ×5 000

16 Cd^{2+}处理 24 h 后的精子 [Sperm 24 h after Cd^{2+} treatment] ×4 000

A: 顶体 (acrosome) GC: 高尔基体 (Golgi complex) L: 溶酶体 (lysozyme) N: 细胞核 (nucleus)



图版说明见文后（Explanation at the end of the text）



图版说明见文后 (Explanation at the end of the text)