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伯氨喹对约氏疟原虫卵囊和子孢子作用的超微结构观察[1]

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Utrastructural study on effect of primaquine on sporogonic stage of *Plasmodium yoelii nigeriensis*

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Abstract Utrastructural changes of oocysts and sporozoites of *Plasmodium yoelii nigeriensis* was observed. *Anopheles stephensi* were allowed to obtain blood meal from the mice which had been adminitered with primaquine diphosphate at different doses and times. Moasquitoes were directed and prepared 6-13 d following infection. Electron microscopy showed that the development and morphology of a number of oocysts and sporozoites in infected mosquitoes after treatment became abnormal. The cytoplasma of the oocysts were partially or totally dissolved and formed vacuoles. The walls of oocysts were thicken. The nuclei and organelles of oocysts and sporozoites were destroyed or damaged. The extent of damage of the oocysts and sporozoites related to the dose of primaquine and the time after the drug administration.

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提要 受染小鼠 ig 伯氨喹 0.25，1.25，1.95 和 2.5 mg/kg 后 2，4 和 8 h 供斯氏按蚊吸血。在感染后 6-13 d 解剖蚊，并以阳性蚊胃作透射电镜观察。给药组的多数卵囊发育不良，其细胞质或形成空泡，或结构疏松，囊壁增厚。卵囊的受损程度与给药剂量和药后感染蚊虫时间的延长有密切关系，有些子孢子的细胞器也受破坏。

关键词 伯氨喹；孢子生殖；卵囊；成孢子细胞；约氏疟原虫尼日利亚亚种；电子显微镜检查

伯氨喹能引起疟原虫红内期和组织期的线粒体发生肿胀，从而使疟原虫组织期发育受阻而被杀灭[1,2]。感染食蟹猴疟原虫(*Plasmodium cynomolgi*)猴口服伯氨喹后 4 h 内蚊吸血能抑制其孢子增殖[3]。但类似实验对疟原虫孢子增殖期的超微结构变化尚未见报道。作者用不同剂量伯氨喹 ig 感染约氏疟原虫尼日利亚亚种(*Plasmodium yeolii nigeriensis*)的小鼠后，于不同时间供蚊吸血，应用透射电镜观察卵囊和子孢子形态结构的变化，以探索伯氨喹对疟原虫孢子增殖期作用的机理。

Materials and methods

约氏疟原虫尼日利亚亚种(*Plasmodium yoelii nigeriensis*)引自英国伦敦热带病研究所 Peters 实验室。昆明杂交系小鼠，体重 20.2±SD 1.8 g，♂，由军事医学科学院动物场繁殖提供。斯氏按蚊(*Anopheles stephensi*)为英国 Hor 株，饲养方法见前文[4]。

磷酸伯氨喹(伯氨喹)由上海第十四制药厂生产。加数滴 Tween-80 研磨，再用无菌蒸馏水配成所需浓度。

实验小鼠给药及感染蚊虫 选择感染率在 20—30%，每 100 个 WBC 视野♀♂配子体数在 20 个以上的小鼠各 4 只供蚊龄 3-4 d 的 300 只♀斯氏按蚊吸血 2 h。吸血前饥饿 18 h，吸血前后饲喂 10% 葡萄糖水，为对照组。取受染小鼠 100 只，匀分为 5 组，分别 ig 0.25，1.25，1.95 和 2.5 mg/kg 的伯氨喹液，并分别于药后 2，4，8 h，随机各取 4 只小鼠供一笼与对照组数量大致相等的蚊虫吸血。其后，上述蚊均置于 25℃ 和相对湿度 75—85% 的养蚊室中饲养。

电镜标本制备与观察 蚊虫感染后于 d 6-13 解剖蚊胃，以 0.2 mol/L，pH 7.0-7.2 的二甲砷酸钠缓冲液配制的 2% 戊二醛为解剖液，将蚊胃置此液中冷固定(4 ℃) 2-3 h，然后用上述缓冲液冲洗数次，直到无戊二醛味为止。保存于 4 ℃，次日至 1 wk 内进行固定、脱水、包埋、切片，用 H-600 型透射电镜进行观察。

Results

正常卵囊的发育及形态 观察 8 批不同龄期(7 和 11 d 龄)正常发育的卵囊。在分化初期卵囊内胞核首先分裂，囊内含多个块状核，同时囊壁内膜下层出现大小不等的空泡(Plate 1-A)，随后空泡渐增大并互相聚合，形成裂隙，且伸向细胞质(Plate 1-B，C)最后将细胞质分隔成大小不等的质块，每一质块再分裂为成孢子细胞，其形态多样。一个卵囊内可含有 1，2 或多个成孢子细胞，由成孢子细胞体长出子孢子芽，逐步形成子孢子(Plate 1-D，E，F)。子孢子外被复合膜，膜下可见长管状微管，前端有一对棒状体及许多微线体(Plate 2-L)。

伯氨喹作用后卵囊和子孢子超微结构变化

1 卵囊 实验组小鼠 ig 不同剂量伯氨喹后，在不同时间感染蚊虫的同期卵囊显示，鼠 ig 0.25 mg/kg 伯氨喹后 2 h 感染蚊虫的 d 7 蚊胃卵囊，大部分发育成正常的早期成孢子细胞，一些卵囊发育不良，在其细胞质中出现大小不等空泡，而且细胞质疏松，呈退化性变化(Plate 1-G)。小鼠 ig 伯氨喹 1.25 mg/kg 后 2 h 感染蚊虫组，少部分卵囊发育正常，大部分卵囊发育不良；药后 4，8 h 感染蚊虫的蚊胃上则难见到发育正常的卵囊；发育异常的卵囊即使形成成孢子细胞，其细胞体出现许多空泡，子孢子芽的胞质也出现空泡(Plate 1-H)。鼠 ig 伯氨

喹 1.95 mg/kg后 2，4，8 h 感染蚊虫，仅 2 h 感染的蚊虫有少量卵囊发育正常，大部分卵囊发育受阻，即使形成成孢子细胞，但子孢子芽也多受破坏，其细胞质结构疏松，并含许多空泡，细胞核与细胞器难以辨认，囊壁增厚(Plate 2- I)。药后 8 h 感染蚊虫的卵囊，全部退化，其细胞质被大小不等的空泡所占据，囊壁增厚(Plate 2- J)。鼠 ig 伯氨喹 2.5 mg/kg 后 2 h 感染蚊虫，蚊胃上卵囊少且全部退化，细胞质出现空泡化较严重，其他细胞器、细胞核和成孢子细胞均未见到(Plate 2-K)。

2 子孢子 鼠 ig 伯氨喹 1.25 mg/kg 后 2 h 感染蚊的胃上，有的卵囊内含有形态不正常的子孢子，其细胞质内出现空泡，有些子孢子的棒状体和微线体融合成团(Plate 2-M, N)。其他给药组(1.95，2.5 mg/kg) 感染蚊虫，所见卵囊未含有发育的子孢子。

Discussion

本实验结果表明，疟原虫孢子增殖与伯氨喹的剂量和受染小鼠服药后感染蚊虫的时间有密切关系。当小鼠服药量少且于短时间内感染蚊，蚊胃上大部分卵囊发育正常，随着药量增加及延长服药后时间感染蚊虫，则发育正常的卵囊数量亦逐渐减少；甚至全部卵囊退化，例如 ig 伯氨喹 1.25 和 1.95 mg/kg 8 h 感染蚊，结果前者仅有个别卵囊发育正常，后者全部卵囊发育受阻，即使形成成孢子细胞，子孢子芽出现空泡，未见发育成熟的子孢子。

感染食蟹猴疟原虫的猴[3]及感染约氏疟原虫配子体的小鼠[5,6]ig 一定量伯氨喹后一定时间内供斯氏按蚊吸血，结果均表明伯氨喹对疟原虫孢子增殖有抑制作用。本实验从超微结构的变化也证实伯氨喹可使部分、甚至全部配子体失去在蚊体内继续发育的能力，即影响卵囊发育和子孢子的形成。其机理如同前人研究结果[1,7]所示，伯氨喹杀灭疟原虫的作用，是由于其代谢产物抑制了疟原虫线粒体的嘧啶合成和摄氧量。本研究结果提示，脊椎动物体内配子体受到伯氨喹代谢产物作用后，受损的线粒体不能进行复原，致使♀♂配子难以受精，即使能发育至卵囊，但也未能形成子孢子。药物剂量的增大和药物作用时间的延长，使卵囊受损现象加重。

本实验的感染小鼠在 ig 1.25 和1.95 mg/kg 伯氨喹 2 h 后感染蚊，可获少量发育正常的子孢子。因此在切断疟疾传染源时，伯氨喹除应注意给予适当药量外，还要留意服药的时间。

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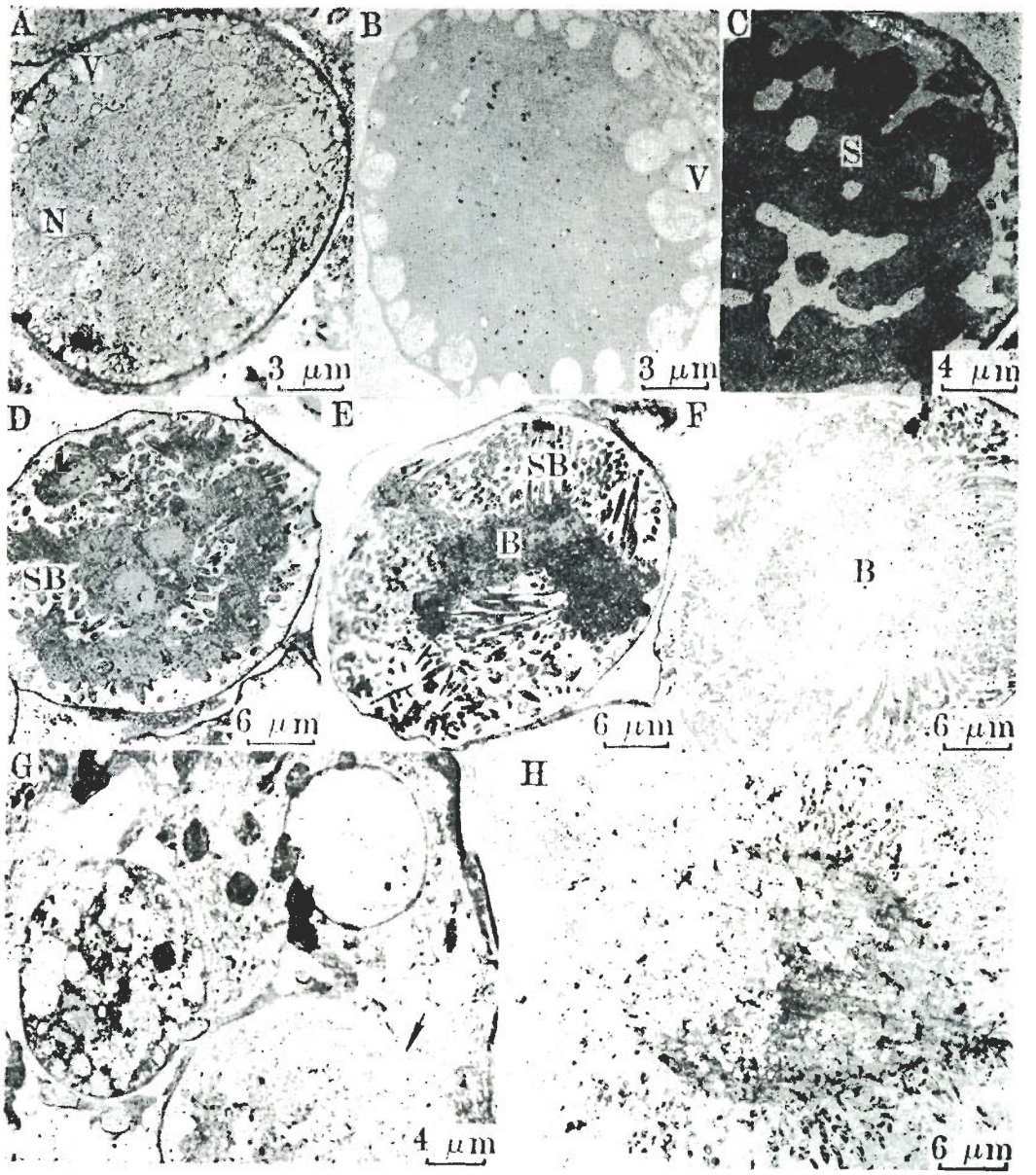

Fig 1. A–D: Normal development of oocyst. A) Early differentiated oocyst showing the divided nuclei (N) and many vacuoles (V) appeared beneath the oocyst capsule. 7 d, ×3000. B) The vacuoles (V) penetrated ever deeper into cytoplasm of oocyst. 7 d, ×3000. C) The cytoplasm of oocyst subdivided and formed sporoblast (S). 7 d, ×2500. D) Early stage of sporoblast, buds (SB) emerged from the surface of the sporoblast body (B). 7 d, ×1500.

E–F: Late stage of sporoblast. E) The sporoblast body (B) was elongated shaped. 7 d, ×1500. F) The sporoblast body (B) was round shaped. 7 d, ×1500.

G–H: The change of oocyst in mosquito midgut infected at different time after mouse ig different doses of primaquine. G) 0.25 mg/kg, 2 h, showing one normal oocyst (arrow) and two vacuolated oocysts, which appeared smaller. 7 d, ×2000. H) 1.25 mg/kg 8 h, one sporoblast in the oocyst, the sporoblast body and sporozoite buds contained many vacuoles. 11 d, ×1500. (See p 283)

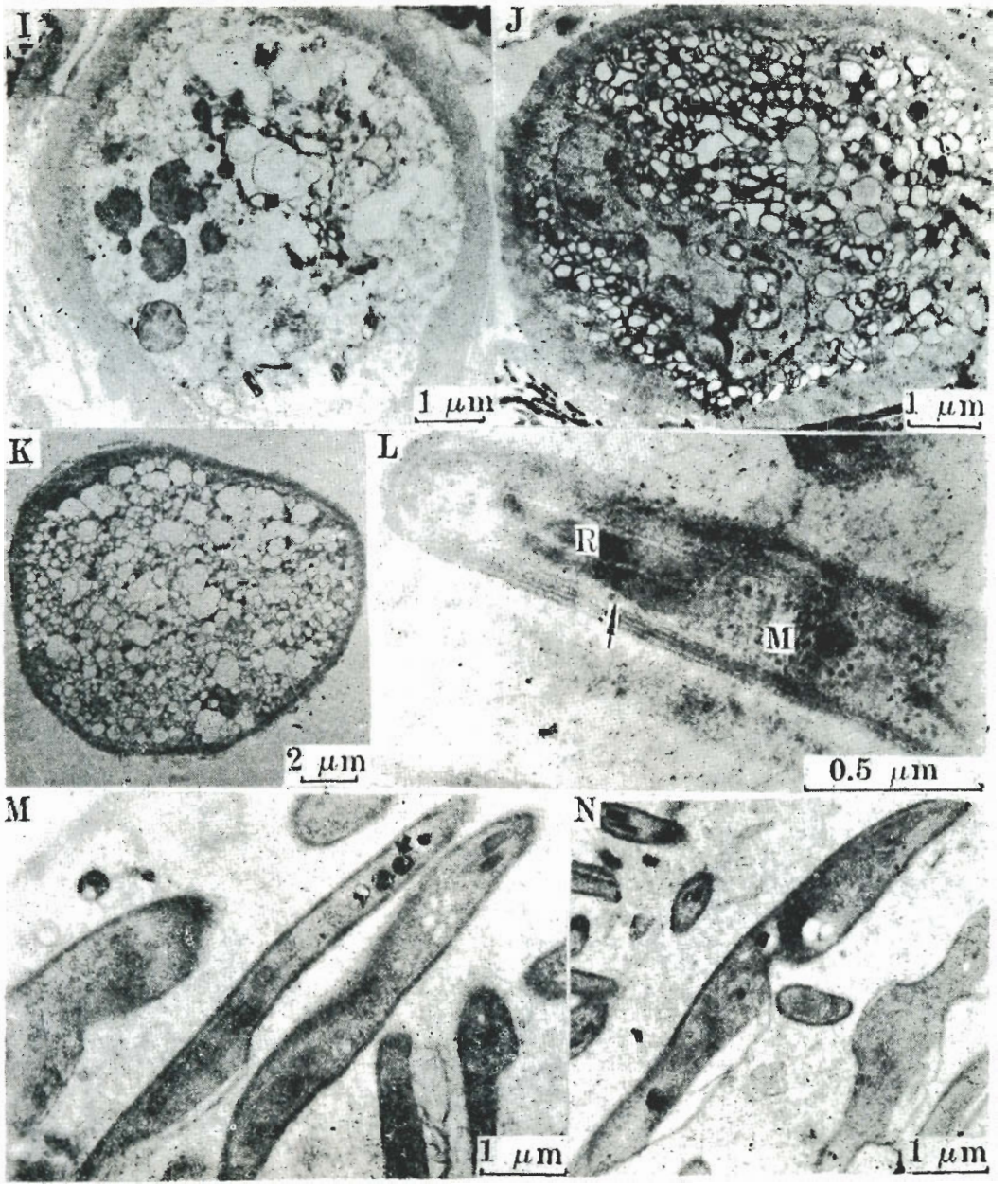

Fig 2. I-N: The change of oocyst. I) 1.95 mg/kg, 2 h, part of the cytoplasm was dissolved while some were massing, vacuoles appeared in oocyst, the wall of oocyst was thickened, 11 d, ×100 00. J) 1.95 mg/kg, 8 h, the cytoplasm of oocyst appeared to contain many vacuoles, the wall of oocyst was thickened, 7 d, ×10 000. K) 2.5 mg/kg, 2 h, the cytoplasm of oocyst filled with vacuoles, 11 d, ×4000. L) Normal sporozoite showing the roptries (R) and many granular micronemes (M), 11 d, ×50 000. M-N) 1.25 mg/kg, 2 h, showing the abnormal morphology of sporozoites, in which appeared vacuoles, the roptries (R) and micronemes were injured, the roptries clustered in lumps (arrow).

(See p 284)