

Some aspects of histological alteration in the tapeworm *Hymenolepis nana* infected white mice by transmission electron microscopy

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Abstract

This transmission electron microscopy (TEM) study has shown that the all of sections were obtained from tapeworm, *Hymenolepis nana* infection which were administered by eggs to white mice at 28 days of age, except in the case of cortisone-treated mice, in which infection was carried out at 38 day of age . The appearance of the brush border does not vary with the age of the worm, nor does treatment with cortisone affect it . The same may be said of distal cytoplasm . There is, however, a dramatic change in the appearance of the perinuclear layer . In older worms (45 day) the number of nuclei is reduced and those which remain are dark in colour . Unidentified structures, which appear to be abnormal, granulated mitochondria, are seen, and lipids accumulate both within and around the cells . Older worms from with cortisone - treated mice also have an altered perinuclear layer, but the changes are different . All of the normal structure are present, but they look as though they are dying because their membranes have a tendency to collapse . As in older worm from hosts which were not treated with cortisone, there was an increase in oil droplets .

Key Words : transmission electron microscope, histological alteration, tegument, mice and tapeworm, *H. nana* .

دراسة بعض مظاهر التحولات النسيجية في الدودة الشريطية المحرشفة القزمة المخمجة
للفئران المهق بواسطة المجهر الالكتروني الامتزازي

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الخلاصة:

تم دراسة مقاطع نسيجية للدودة الشريطية المحرشفة القزمة باستعمال المجهر الالكتروني الامتزازي والتي تم الحصول عليها من اخماج الفئران البيض عن طريق تجريعها ببويض الديدان في عمر 28 يوما (ما عدا الفئران المحقونة بعقار الكورتيوزون فقد كان تجريعها بعمر 38 يوما) . لقد اظهرت نتائج فحص المقاطع النسيجية للدودة ان الحافة الاجمية للطبقة الشعرينية (الخارجية) للدودة لم تختلف بتقدم عمرها او التي عوملت عوائلها بعقار الكورتيوزون وان طبقة الهيولي القاصي او ما تسمى الجليدة الاساسية (الطبقة الوسطى) لم تختلف كثيرا هي الاخرى ، في حين ان هناك تحولات لافتة في بنية الطبقة الداخلية (او ما تسمى حول النووية) اذ يختزل فيها

عدد الانوية كثيرا (خصوصا الديدان الهرمة بعمر 45 يوما) وما تبقى من هذه الانوية فيظهر بلون داكن ، وقد شوهدت تراكيب غير سوية منها المتقدرات الحبيبية والتراكبات الدهنية داخل وحول الخلايا . ان الطبقة الداخلية للديدان الهرمة والتي عوملت عائلها (الفئران) بالكورتيزون حدثت فيها تحولات مختلفة نوعا ، اما التراكيب السوية فهي موجودة ولكنها تبدو وكأنها ميتة بسبب ميل اغشيتها نحو الانكماش ، كما ان هناك زيادة في القطيرات الدهنية في الطبقة الداخلية للديدان الهرمة والتي لم تعامل عائلها من الفئران بالكورتيزون .

Introduction

H. nana commonly known as the dwarf tapeworm, is cosmopolitan endoparasite and abound in the tropical climates (1) . Cestode surface has been the subject of much investigation in the last two decades . Since cestodes lack mouth or digestive system they have a metabolically active surface through which nutrients can be absorbed and waste materials eliminated (2) . The cestodes tegument is however, unique in bearing microvilli (microtriches), consisting of cylindrical cytoplasmic bases capped by dense structures termed, shaft (3) . Beneath the microtriches lies a layer called distal cytoplasm that contains abundant vesicles and electron - dense bodies as well as numerous mitochondria . The distal cytoplasm is connected to cytons by channels or internuclear processes that run through the superficial muscle layer . Nuclei lie in the cytons, not the distal cytoplasm (4) . The expulsion of *H. nana* from the small intestine of the mouse is due to an immune response (5) . It therefore seemed of interest to examine the tegument of worms at different ages . The aim of this study has involved TEM to detect any histological changes in different layers of worm integument .

Materials and Methods

Mice

Male albino Balb / c – mice each 8-10 weeks and weighing 20-25 gm (from the Animal House at Al-Nahrain University) .

Parasite

The parasite strain was originally obtain from Dr. A . H . Jasim at the

College of Veterinary Medicine / Baghdad

Infection of mice with *H. nana* eggs by stomach tube

Gravid proglottids obtain from an early already – infected mouse were transferred to a pestle containing normal saline, and ground gently with a mortar . The egg suspension was allowed to stand for a few hours and then it was withdrawn carefully by pipette, preventing the sediment from mixing with the supernatant . The egg suspension was then shaken vigorously and three 0.1 ml aliquots were taken by graduated syringe ; each 0.1 ml was placed on a clean slide and the eggs were counted in each aliquot . The mean was taken and dilutions made to any required concentration of eggs (9) . The solution was then given to the mice by stomach tube . The infected mice were killed after 13 – 15 days . The intestine was opened in a Petri dish filled with normal saline . The worms allowed to relax .

Injection of mice with cortison acetate

Mice were injected subcutaneously with 0.025 ml of cortison acetate three time a week . They were given Terramycin (soluble powder) with their drinking water by dissolving 0.5 gm in one liter of water (6) .

Transmission electron microscopy

Approximately 0.5 cm portions of *H. nana* were removed from mice immediately after they had been killed, and were fixed for 2 hours in 25% glutaraldehyde in sodium cacodylate buffer (pH 7.4) . The tissue was washed in

buffer and post fixed in Osmium Tetraoxide solution (4% OsO_4) and stained with uranyl acetate, washed in malate buffer (pH 5.2) , and then dehydrated in ethanol and propylene oxide and embedded in Araldite . Section were cut with glass knives in Huxley ultramicrotome, and mounted on copper grids and stained in lead citrate, then observed under a Philips 300 transmission electron microscope (9) .

Results

All of the sections were obtained from worms in infections which were administered by eggs, to mice at 28 days of age, except in the case of cortison – treated mice, in which infection was carried out at 38 days of age . The sections show that the normal body covering has three distinct layer (fig.1) . The first is that of microtrichia, which form a brush border . The second is the distal cytoplasm, some times called the true cuticle . It contains a variety of granules scattered throughout the cytoplasm, in the mitochondria . The inner layer, or perinuclear layer, is more complex . It comprises many kinds of cells,

and these in turn contain the usual complement of nucleolus, nucleus, mitochondria, Golgi apparatus, endoplasmic reticulum, and also glycogen granules and oil droplets . There are muscle fibres within this layer . The appearance of the brush border does not vary with the age of the worm (fig . 2), nor does treatment with cortison affect it (fig . 3) . The same may be said of the distal cytoplasm (fig . 4) . There is, however, a dramatic change in the appearance of the perinuclear layer . In older worms (45 days, fig . 5), the number of nuclei is reduced . Those which remain are dark in colour . Unidentified structures which appear to be normal, granulated mitochondria, are seen, and lipids accumulate both within and around the cells . Older worms from cortison – treated mice also have an altered perinuclear layer, but the changes are different (fig . 6 , fig . 7) . All of normal structures are present but they look as though they are dying because their membranes have a tendency to collapse . As in older worms from hosts which were not treated with cortison, there was an increase in oil droplets .

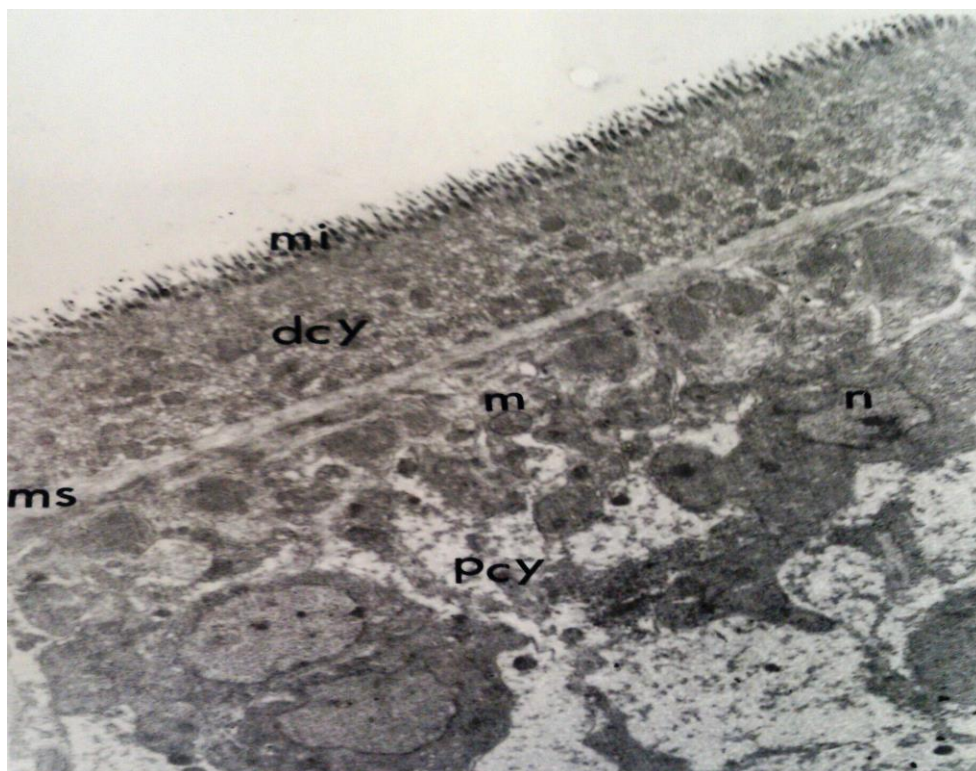


Figure 1: Section from a 9-day-old worm (fixed in glutaraldehyde, stained with uranyl acetate and lead

citrate) . The three layers of the body surface can be clearly seen. Magnification X4350.
(mi : microtrichia , dcy : distal cytoplasm , ms : muscle , pci : parenchyma , n : nucleus ,
m : mitochondria) .

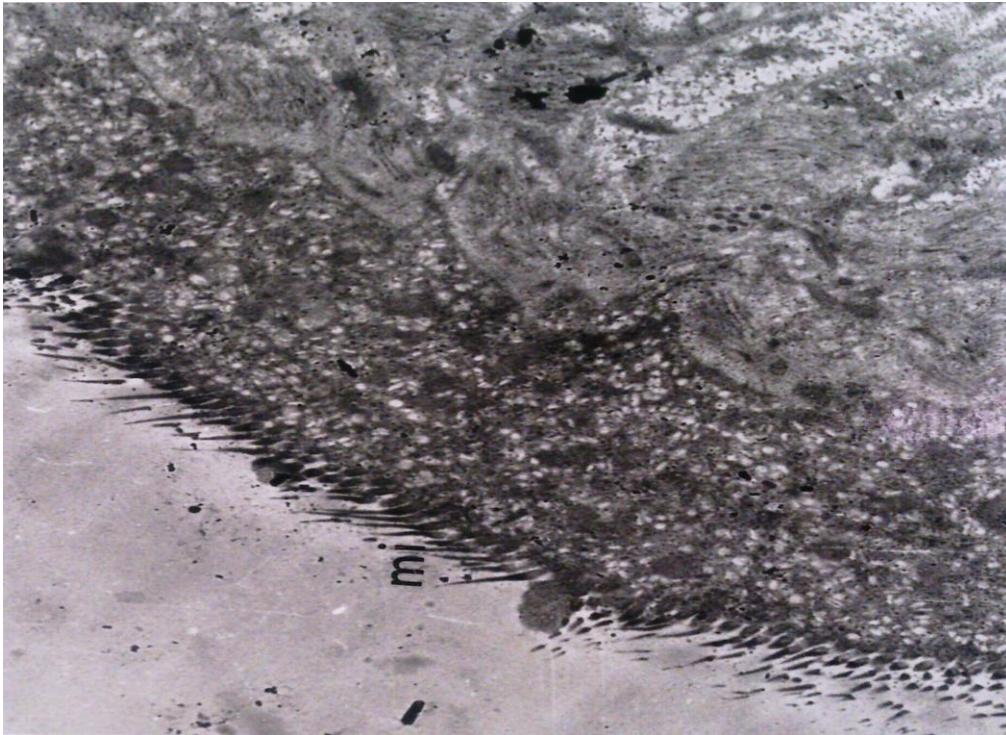


Figure 2 : Section from a 45-day-old worm (glutaraldehyde, uranyl acetate and lead citrate) . Note that the brush border has a normal appearance. Magnification x4300, (mi : microtrichia) .

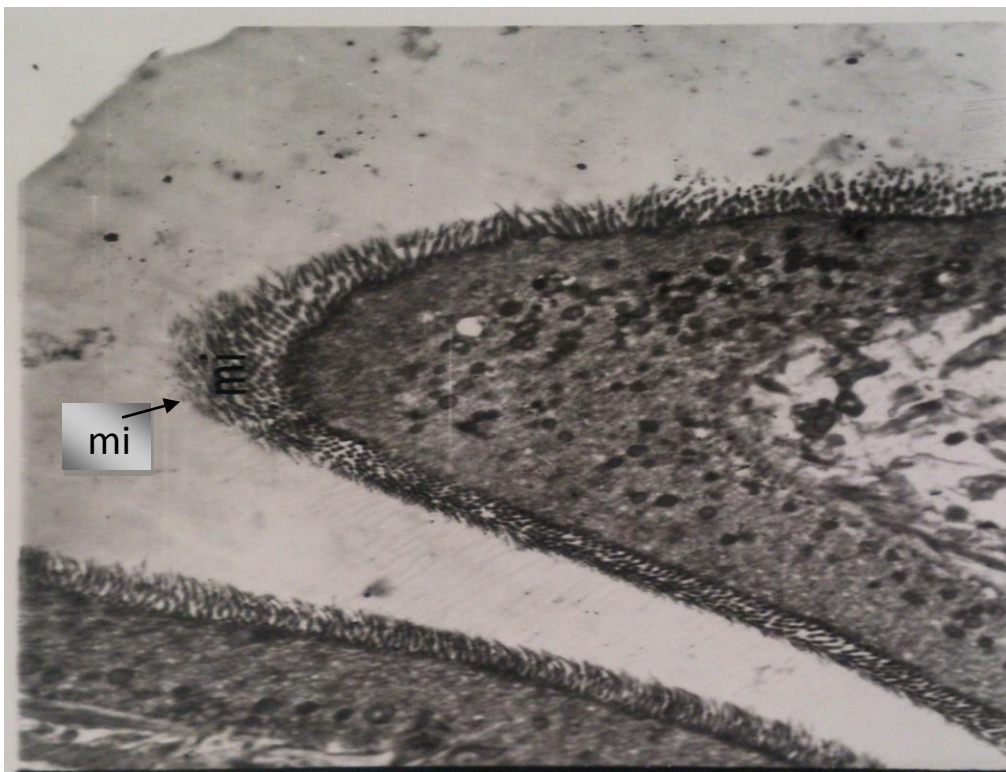


Figure 3 :Section from a 34 – day – old worm from a cortisone-treated mouse (glutaraldehyde,

uranyl acetate and lead citrate). Here too the brush border appears normal. Magnification x4640, (mi : microtrichia).

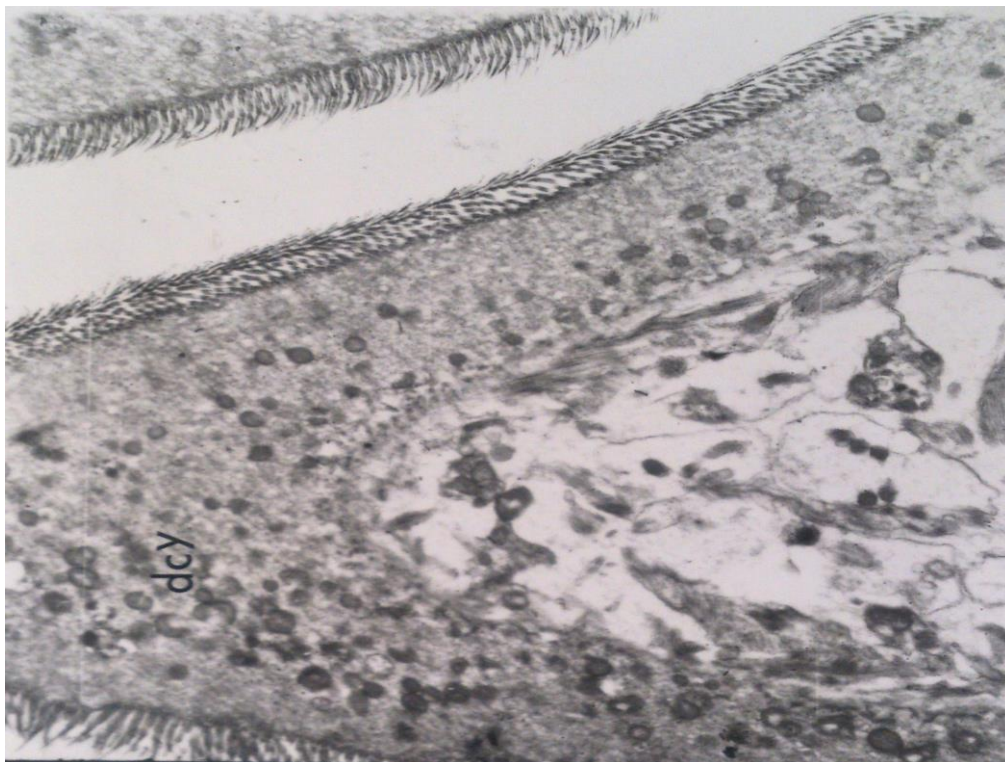


Figure 4: Section from a 34 - day - old worm from a cortisone-treated mouse (glutaraldehyde, uranyl acetate and lead citrate), showing how the distal cytoplasm has a normal appearance. Magnification x8170, (dcy : distal cytoplasm) .

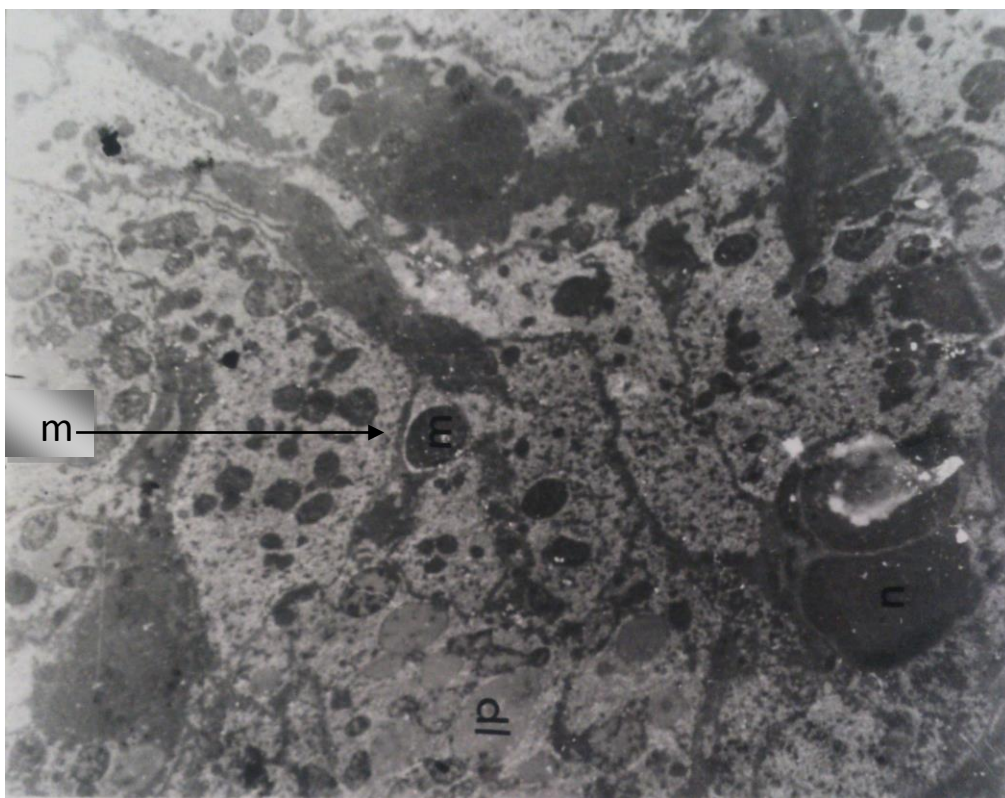
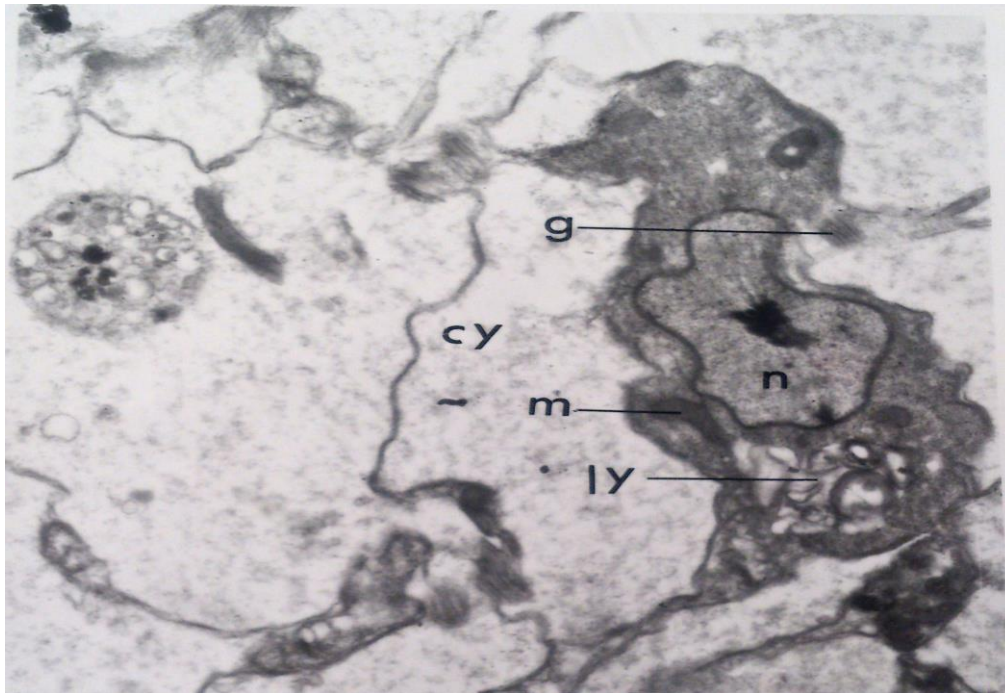


Figure 5: Section of a 45-day-old worm (glutaraldehyde, uranyl acetate and lead citrate). Note the

disappearance of many cell contents, the dark nucleus(n), abnormal mitochondria (m) and accumulation of lipids . Magnification x 12600, (lp : lipids) .



Figure

6 : Section from a 34 – day - old worm a cortison – treated mouse (glutaraldehyde, uranyl acetate and lead citrate) . Note the damage to the cells, and the lysosomes with collapsed membranes . Magnification x 12600, (g : Golgi body, cy : cytoplasm, m: mitochondria , n : nucleus , ly : lysosome) .



Figure 7: Section of a 34 – day – old worm (glutaraldehyde, uranyl acetate and lead citrate). Note the apparently normal brush border and distal cytoplasm, but damaged perinuclear layer with accumulation of oil droplets. Magnification X5800. (ly : lysosome, lp : lipid, n : nucleus, m : mitochondria).

Discussion

Structural changes to parasites within the gut which appear to be a result of host immunity have been described by a number of workers, but less work has been carried out with tapeworm, *H. nana*. The most important paper is that of (7), who showed that immunological damage can occur in *H. diminuta*. This includes increasing amount of lipids, abnormal mitochondria, and dark areas on the surface of worms which had been incubated in Hank's saline. The results presented here confirm some of (8), but they also add much new information. First of all, it is clear that the tegument cells become damaged. Normal tegumental cells are readily recognized by their dense granulated nucleus and cytoplasm; they usually lie quite deep within the parenchyma and are probably isolated from one another. They usually contain distinctive structure (9). It is the disappearance of these, together with the abnormal mitochondria and the accumulation of lipids which indicates that the cells in older worms which are exposed to an immune response are in poor condition. The accumulation of lipid in particular indicates injury (10) and (11). In nematodes, (12), (13) explained the accumulation of lipid as being caused by a reduction in available oxygen as the worms were driven from the mucosa into the lumen of the gut. (7) and (14) suggested that lipid accumulation was due to metabolic changes in the mitochondria which are morphologically abnormal.

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