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SHORT PAPER

Copper localization in the duodenal mucosa of heterozygous tortoiseshell ($Mo^{to}/+$) female mice (*Mus musculus*)

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SUMMARY

The duodenal mucosa of normal (+/+) and heterozygous tortoiseshell ($Mo^{to}/+$) female mice was analysed by an ultrastructural histochemical copper localization technique. Copper was rarely detected along the duodenal microvilli of +/+ female mice. However, copper was localized on the surface of the brush border of the duodenal mucosa and within pinocytotic vesicles at the base of the microvilli of $Mo^{to}/+$ female mice. The mottled defect may involve a defective intestinal uptake of copper, as well as a faulty copper transport mechanism.

1. INTRODUCTION

The tortoiseshell (Mo^{to}) gene, which arose spontaneously in an obese stock as a heterozygous female (Dickie, 1954), is an allelic form of the mottled locus (Lane, 1960). Mottled mice exhibit an intestinal copper transport defect, altered coat pigmentation, and various associated copper metabolic disorders (Dickie, 1954; Hunt, 1974; Hunt, 1976; Hunt & Port, 1979; Silvers, 1979).

An increased intestinal copper content has been detected in brindled mice (Camakaris, Mann & Danks, 1979; Prins & Van den Hamer, 1979) and $Mo^{to}/+$ female mice (Sheedlo, unpublished). Various investigators have postulated that the mottled defect does not involve uptake of copper from the intestinal lumen but rather a release of copper from those cells implicated in copper transport (Hunt, 1976; Prins & Van den Hamer, 1979; Mann, Camakaris & Danks, 1980). However, copper was detected by Horn & Jensen (1980) concentrated on the brush border of the duodenum of Menkes' patients, a homologue of the sex-linked mottled locus in mice. These results seem to indicate that the Menkes' and mottled defects involve both the uptake and transport of copper.

The principal aim of this study was to determine the site of copper concentration in the duodenum of $Mo^{to}/+$ female mice. In addition, a theory of the mottled defect was developed correlating the results of this study and separate studies of $Mo^{to}/+$ female mice and the other mottled alleles.

2. MATERIALS AND METHODS

(*i*) *Animals*

The +/+ and Mo^{to}/+ female mice used in this study were offspring of C57BL/6J males mated with C57BL/6J-Mo^{to}/+ females. The initial breeding stock was purchased from Jackson Laboratories, Bar Harbor, Maine. All mice were about 3 months of age. The animals were housed in plastic cages with steel tops and provided with Purina Laboratory Chow (15·0 ug copper/gram weight pellet) and tap water *ad libitum*.

(*ii*) *Copper localization*

The duodenum of anaesthetized +/+ and Mo^{to}/+ female mice was quickly removed and minced in 2·5 % glutaraldehyde in 0·1 M cacodylate buffer, pH 7·0. Following a 2-h fixation, the minced tissues were stained for copper by a modified method of Scheuer, Thorpe & Marriott (1967). The tissues were either post-fixed in 2·0 % osmium tetroxide or remained unosmicated, dehydrated in an ascending acetone series, and embedded in Epon (Scheuer *et al.* 1967; Horn & Jensen, 1980). The tissues were sectioned on a Porter–Blum MT2-B ultramicrotome with the gray-to-silver sections placed on uncoated 200 mesh gold grids. Post-stained (Venable & Coggeshall, 1965) and unstained sections were examined on a Zeiss EM-10 transmission electron microscope.

3. RESULTS

Insignificant quantities of copper were detected along the duodenal microvilli of +/+ female mice; however, limited amounts of copper occurred within pinocytotic vesicles of the duodenal epithelium (Plate 1, fig. 1).

Copper was detected all along the surface of duodenal microvilli and within pinocytotic vesicles at the bases of microvilli of Mo^{to}/+ female mice (Plate 1, fig. 2). Copper was extensively concentrated at the apex and base of each microvillus (Plate 1, fig. 2, Plate 2, fig. 3). Numerous pinocytotic vesicles containing copper were found within the duodenal mucosa of Mo^{to}/+ female mice (Plate 2, fig. 4).

DISCUSSION

The results of this ultrastructural copper investigation of the duodenum of Mo^{to}/+ female mice parallel the study of Horn & Jensen (1980) concerning the Menkes' disease. Increased duodenal copper has been found in Mo^{to}/+ female mice (Sheedlo, unpublished) and thus supports the contention that the granules detected at the ultrastructural level represent copper.

Crampton, Matthews & Poisner (1965) concluded that copper absorption by the hamster small intestine does not involve a diffusion process. Pinocytotic vesicles containing copper (Plate 2, figs 3, 4) may be the major mode of copper transport across the intestinal mucosa. This mechanism of copper transport would be energy dependent.

The copper-binding protein, metallothionein (copperthionein) may function in regulating the transport of copper by the intestinal mucosa in both the pinocytotic uptake and transfer of copper to the portal blood (Johnson & Evans, 1980). Copper not transported across the mucosa would be lost in the faeces by desquamation of the intestinal epithelial cells at the apex of the microvilli.

Many theories of the mottled and Menkes' defect have been proposed (Hunt, 1974; Hunt, 1976; Prins & Van den Hamer, 1979; Horn & Jensen, 1980; Prins & Van den Hamer, 1980). The mottled defect possibly involves an altered copperthionein protein which has a reduced copper-binding capacity (fewer copper-binding sites per molecule) and a diminished intestinal absorptive capacity. Copper localized along the intestinal

brush border of $Mo^{to}/+$ female mice would result from reduced copper absorption. Also, increased synthesis of the altered copperthionein would be necessary compared to the unaltered protein to bind an equivalent copper level. Prins & Van den Hamer (1980) observed an increased metallothionein concentration in the kidney of brindled males and concluded that the increased metallothionein levels may account for increased copper retention by the kidney. Increased lysosomal activity in the kidney of $Mo^{to}/+$ female mice (Sheedlo, unpublished) may be a consequence of the increased levels of the altered copper-binding protein.

Portions of this study will be submitted in partial fulfillment of the Doctor of Philosophy Degree.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Electron micrograph of the duodenal microvillar region of a + / + female mouse. Vesicles are observed containing a small number of silver granules (arrows) (× 39200). MV, Microvilli, L, Lumen.

Fig. 2. Electron micrograph of the duodenal microvillar region of a $Mo^{to}/+$ female mouse. Copper is localized on microvilli and within pinocytotic vesicles (arrows) (× 39000). MV, Microvilli, L, Lumen.

PLATE 2

Fig. 3. Electron micrograph of the duodenal microvilli of a $Mo^{to}/+$ female mouse (×122500). MV, Microvilli, L, Lumen.

Fig. 4. Electron micrograph of the bases of duodenal microvilli of a $Mo^{to}/+$ female mouse (×49000). MV, Microvilli. Arrows indicate copper-containing pinocytotic vesicles.

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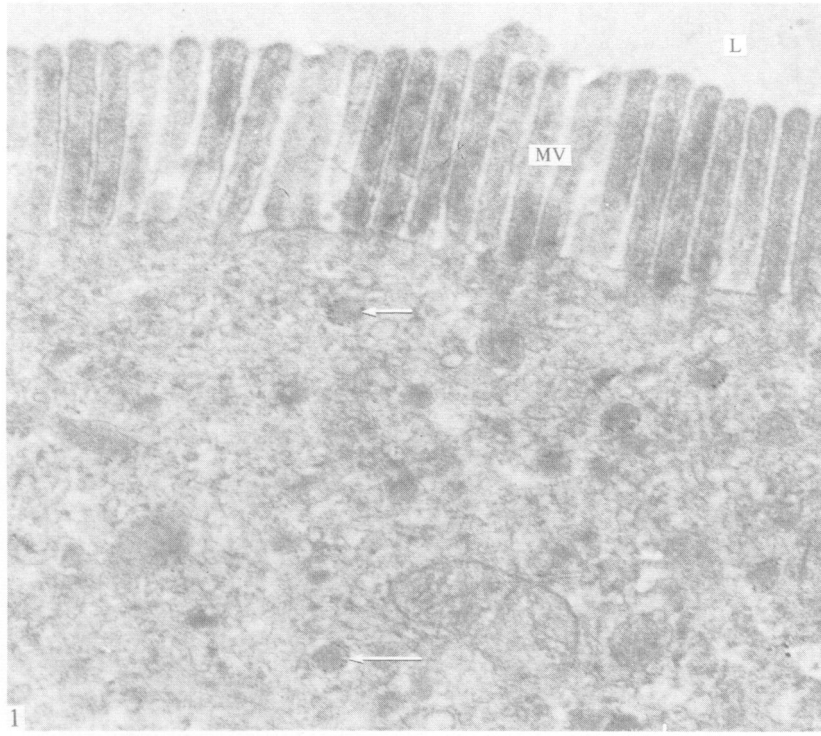

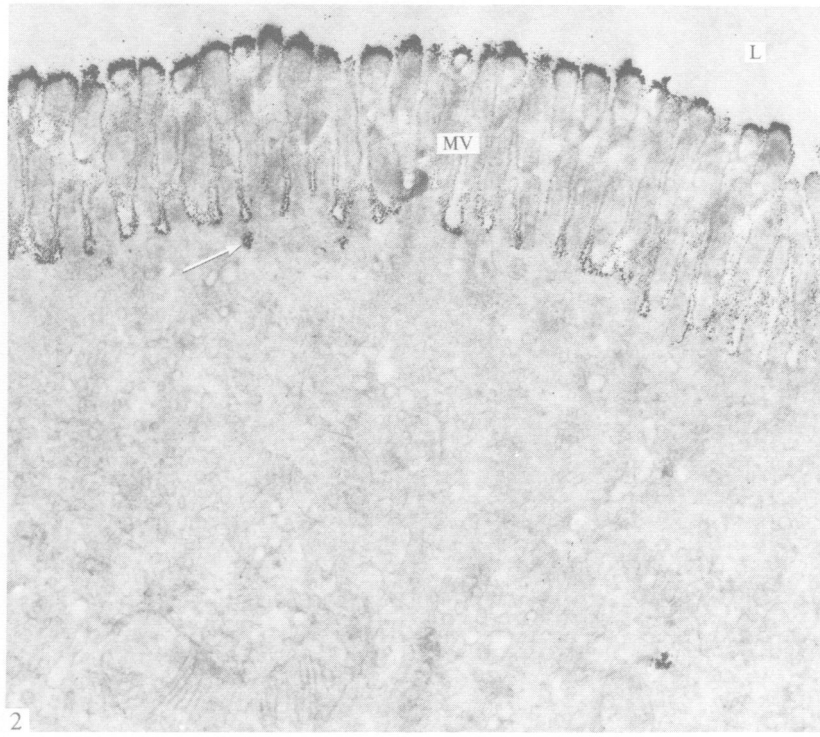

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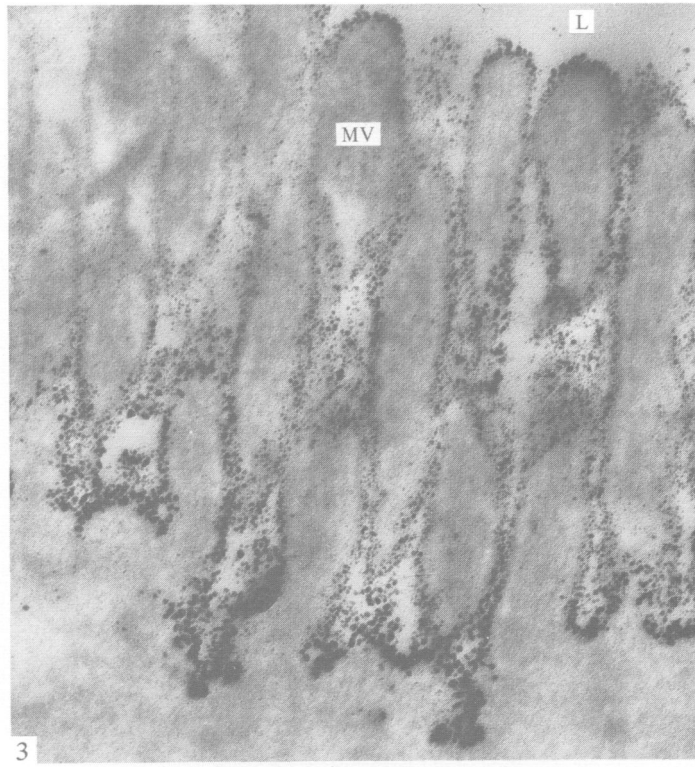

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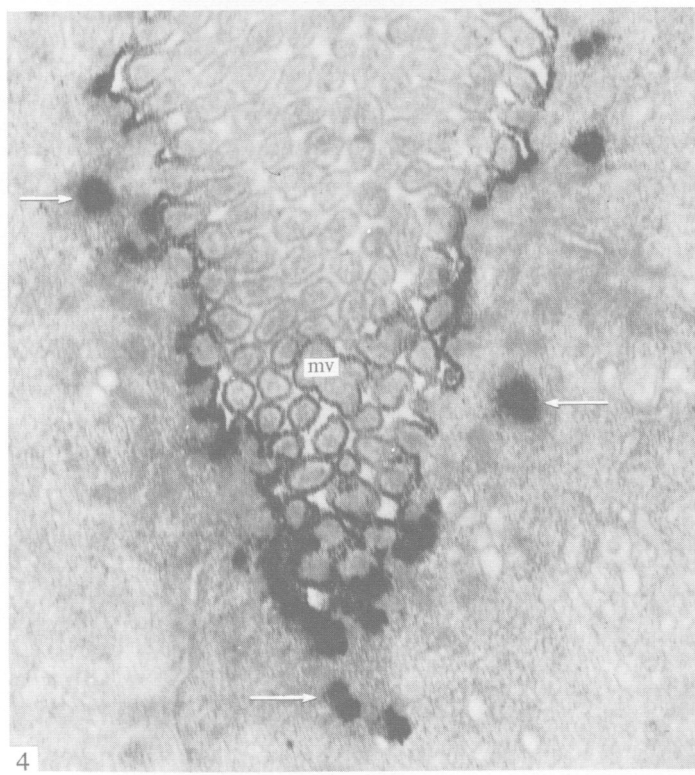

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