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Manganese Induced Histochemical and Histological Alterations in Gastrointestinal Mucosa of Guinea Pigs

By

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Abstract: Manganese chloride (10 mg/kg) was administered orally to guinea pigs for a period of 30 days. The histochemical and histological alterations in gastric mucosa consisted of loss of mucin and pepsinogen granules, adenosine triphosphatase and glucose-6-phosphatase activities and patchy necrosis of the epithelium. The intestinal villi also showed patchy necrosis, the activities of adenosine triphosphatase and glucose-6-phosphatase were diminished while there was an increase in the activity of acid phosphatase in the mucosa. Excess of manganese in the gastrointestinal tract produces functional and structural alterations in the mucosal cells. The exact mechanism of action needs further study.

Key-words: Manganese toxicity - gastrointestinal tract.

Manganese is known to produce neurological disorders when inhaled by miners and the lung has long been thought as the main route of entry of this metal (COUPER 1837; RODIER 1955; TANAKA & LIEBEN 1969). Recently it has been shown that the inhaled manganese finds its way into the gastrointestinal tract from which it is either absorbed or eliminated (MENA et al. 1969). Irrespective of the mode of entry the importance of the gastrointestinal tract in manganese toxicity assumes great significance.

Administration of large doses of manganese salt into the stomach of animals produces corrosion of the gastric walls and the intestines (VON OETTINGEN 1935). In view of the paucity of the literature and the importance of the gastrointestinal tract in manganese toxicity, histological and histochemical studies of the gastrointestinal mucosa were undertaken in guinea pigs after oral administration of manganese chloride.
Materials and methods

Seventy male guinea pigs of average weight 350 g from I. T. R. C. colony were divided into three groups. Group I consisting of 20 animals were kept as normal controls. Group II consisting of 20 animals were given orally with a cannula 1 ml physiological saline daily for a period of 30 days and group III consisting of 30 animals were given orally manganese chloride (10 mg/kg) in a similar manner daily for the same period.

Two animals from group II and six animals from group III died during the course of the experiment. The dead animals were autopsied but the cause of death could not be ascertained. The remaining animals were sacrificed at the end of the experiment by ether anaesthesia. The animals were chilled on crushed ice, and the stomach and intestinal segments were taken out and rinsed with ice-cold solution of 5% sucrose. The stomach was cut open along the greater curvature and small pieces from different parts were cut out. Approximately 1 cm long segments were cut from the jejunum, ileum and colon. These tissues were treated in three different ways. (a) Small pieces were fixed in chilled 1% calcium formol solution for 24 hrs at 4°, rinsed with 5% sucrose, blotted gently and put in gum sucrose for 24 hrs at 4°, after which free floating sections 10-15 μ thick were cut in a freezing microtome. (b) Unfixed segments were frozen in liquid nitrogen and directly mounted on to microtome chucks and kept at −20° until sections 5-6 μ thick were cut in cryostat; the sections were then mounted on cover slips for enzymic studies. (c) Small pieces of stomach were fixed in Regaud’s fixative (McMANUS & MOWRY 1965).

The remaining stomach specimens were placed on stiff paper and together with the remaining segments of intestines were fixed in neutral buffered formalin. The tissues were dehydrated in graded alcohol, cleared in toluene and embedded in paraffin. Sections were then cut and stained with haematoxylin and eosin, PAS, alcian blue (McMANUS & MOWRY 1965), and for pepsinogen granules, by Bensley’s neutral gentian stain (Cowdry 1948). Other histochemical methods included the demonstration of alkaline and acid phosphatases (GOMORI 1939 & 1941), adenosine-triphosphatase (ATPase) by the method of Padykula & Herman (1955) and glucose-6-phosphatase by the method of Wachstein & Meisel (1956). For comparison, sections of the same region of the gastrointestinal tract of control animals were studied for enzymic activity. Controls were also studied for each enzyme by incubation in the respective incubation media without specific substrate and at the same time one cryostat section from each block was stained with haematoxylin and eosin.

Results

Macroscopic. No gross abnormality was observed on the serosal surface of the stomach in all the groups. After cutting open along the greater curvature, the mucosa was found to be greyish pink in colour with numerous folds in groups I and II. A few small pin point haemorrhages and abrasions were noticed along the greater curvature of the stomach in group III. The intestines did not show any gross abnormality.

Microscopic. The gastric and intestinal architecture in groups I and II was normal and almost resembled the histology of the gastrointestinal tract described by Bloom & FawCet (1966).
The chief cells which were present in the lower half of the gastric mucosa were filled with pepsinogen granules and the mucous cells were distended with mucin.

**Stomach.** In group III patchy morphological changes were observed throughout the mucosa. The surface epithelial cells were ragged with a variable intensity of nuclear staining and irregular positioning of the nucleus within the cells, in some places the superficial epithelium was totally absent. The glands were atrophic and distorted with reduced number of cells. Some of the cells were lying free and the nests of glandular cells were no longer attached together in an orderly arrangement. In some places, degenerated mucosal cells with pyknotic nuclei and homogenous eosinophilic cytoplasm were seen. There was oedema of the interglandular tissue with congested blood vessels and fine connective tissue. Red blood cells were seen lying free in the interglandular spaces. The lamina propria showed dilated and congested blood vessels, with infiltration of round cells (fig. 1). The pepsinogen granules were absent from most of the chief cells (figs. 2 and 3) and mucous cells also showed marked depletion of mucin granules as was evident from their histochemical reactions.

**Adenosine triphosphatase.** In the animals of groups I and II marked activity of ATPase was observed in the supranuclear and along the lateral cell membrane of the superficial epithelial cells. Mild activity was seen in the glandular cells in both the fundus and pyloric regions. In group III
very faint activity of ATPase was observed in the supranuclear and along the lateral cell membrane of the glandular cells. In some places the activity was totally absent.

\textit{Glucose-6-phosphatase}. In groups I and II marked activity of glucose-6-phosphatase was observed in the gastric mucosal cells. The reaction was diffuse throughout the cell cytoplasm. In manganese fed animals the activity of this enzyme was very faint in the cytoplasm of the mucosal cells, and in some places activity was totally absent.

Acid phosphatase activity was not demonstrated in the cells of the gastric mucosa. There was no change in the activity of alkaline phosphatase in the manganese treated animals.

\textit{Small intestines}. In the animals of group III the mucosa of the jejunum and ileum showed flattening of villi. In some places villi were absent, the surface epithelium was denuded, the nuclei were pyknotic and there was a

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image}
\caption{Gastric mucosa of control guinea pig showing pepsinogen granules in the chief cells. Bensley's neutral gentian stain. Magnification $\times680$.}
\end{figure}
collection of inflammatory cells. The lamina propria contained inflammatory cells namely lymphocytes, plasma cells and histiocytes (fig. 4). The glandular epithelium was very much narrowed and there was focal involvement of glandular epithelial cells showing degeneration and necrosis.

*Acid phosphatase.* Acid phosphatase activity in the group I and II animals was located in the cytoplasm of the superficial epithelial cells and in the histiocytes in the lamina propria in the mucosa of jejunum and ileum. In the animals of group III the acid phosphatase activity was seen in the cytoplasm of surface epithelial cells. The histiocytes in the lamina propria showed a very intense reaction for this enzyme (figs. 5 and 6). No significant alteration in the activity of alkaline phosphatase was observed in the epithelial cells of villi in all the groups.

*Adenosine triphosphatase.* In the animals of groups I and II the brush
border and deeper glands showed intense activity of ATPase. In the mucosa of the group III animals, patchy loss of activity of ATPase was observed in the brush border and deeper glandular cells, while in some places there was no activity.

Fig. 4. Jejunal epithelium of guinea pig showing flattened and necrosed villi with inflammatory reaction. Haematoxylin and eosin. Magnification ×300.

Fig. 5. Jejunal villi of control guinea pig showing acid phosphatase activity in the supra-nuclear region of the cells and in the histiocytes in the lamina propria. Magnification ×600.
Fig. 6. Acid phosphatase activity in jejunal villi of guinea pig after oral administration of manganese for thirty days; the localisation of enzyme activity is similar in the surface epithelial cells but the number of acid phosphatase containing histiocytes in the lamina propria is very much increased. Magnification $\times 600$.

*Glucose-6-phosphatase.* In the animals of groups I and II, superficial mucosal cells, cells covering the sides of villi, crypts and deep glandular cells reacted intensely for glucose-6-phosphatase activity. There was uniform

Fig. 7. Glucose-6-phosphatase activity in the mucosa of control guinea pig ileum showing intense reaction in the mucosal cells. Magnification $\times 375$. 
reaction in the cytoplasm. In the mucosa of the group III animals a very faint reaction of glucose-6-phosphatase was seen in the superficial cells, cells covering the sides of villi, crypts and deep glandular cells. In some places no activity was observed (figs. 7 & 8).

*Large intestines.* No pathomorphological alterations were observed in the large intestines.

**Discussion**

Histological changes in the gastrointestinal tract are known to occur with chemicals like salicylates, phenylbutazone and salts of copper and cobalt (Plantevydt & Willighagen 1960; Zaidi & Mukerji 1962; Browning 1969; Chandra & Singh 1967). In our experiments oral administration of manganese chloride in guinea pigs for thirty days has resulted in patchy necrosis of the mucosa of the stomach and small intestines. Necrosis of the bronchial mucosa after intratracheal administration of manganese chloride in rats was observed by Davies & Harding (1949). The histochemical alterations showed a marked decrease in mucin and pepsinogen granules in the gastric mucosa and in the activities of adenosine triphosphatase and glucose-6-phosphatase in the mucosa of the stomach and small intestines.
The disturbed cellular functions as evident from histochemical changes are not in proportion to the histological evidence of cellular damage in the mucosa. Similar disproportion in the functional state and histological changes in gastritis have been observed by Rohner & Welsh (1967). Manganese may produce structural changes not appreciable by light microscopy, thus inhibiting the cellular functions or in the first instance it may alter the cellular functions rather than produce actual destruction.

The significance of the intracellular mucosal enzymes in gastrointestinal physiology is poorly understood. The presence of ATPase in the gastric and intestinal mucosa is probably concerned in the release of energy required for the absorption, transport and synthetic activity of the cell. Glucose-6-phosphatase helps in the metabolism of carbohydrate and finally in the absorption of glucose in the blood stream. Thus the loss of ATPase and glucose-6-phosphatase activity in the gastrointestinal mucosa in our experiments indicates impairment of metabolic activity of the cell under the toxic influence of manganese. A decreased activity of ATPase in brain due to manganese toxicity has been reported previously (Chandra 1972). Inhibition of ATPase activity also occurs in the nephrons of experimental animals after the intravenous administration of manganese chloride (Jonek et al. 1965).

The activity of acid phosphatase was not demonstrable in the stomach. The absence of acid phosphatase activity in the mucosa of the stomach of dogs, rats and cats was also reported by Gomori in 1941 (loc. cit.). The increase in the activity of acid phosphatase in the histiocytes of the lamina propria in manganese treated animals may be due to an increased histiocytic reaction resulting from an excess of manganese. An increase in acid phosphatase activity has been observed in the kidneys and cerebellum of rabbits intoxicated with manganese (Jonek et al. 1965, loc. cit.; Jonek et al. 1966). The activity of alkaline phosphatase was not affected by manganese as has been observed in our previous studies on the brain (Chandra 1972, loc. cit.). The present investigations have shown that the excess of manganese in the gastrointestinal tract produces functional and structural alterations in the mucosal cells. The exact mechanism of action remains to be elucidated.

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