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THE LOCALIZATION OF TETRACYCLINE IN THE METASTATIC CALCIFICATIONS IN THE STOMACH OF RAT INDUCED BY OVERDOSAGE OF DIHYDROTACHYSTEROL AND VITAMIN D_3

By

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Tetracycline, owing to its strong yellow fluorescence, is easily detected in tissues. It has been observed that tetracycline combines itself with malignant growths (*Rall et al.* 1957) and especially with the mitochondrias (*DuBuy & Showarce* 1961). The localization of tetracycline in bone tissue is very stable, lasting for weeks (*Mich et al.* 1957). However, a more intense fixation is found in the growing new bone than the old (*Harris et al.* 1962). *Häkkinen* observed (1958) that tetracycline combines itself with the metastatic calcifications produced by dihydrotachysterol in rats, and DOCA was found to intensify the reaction, but cortisone, on the other hand, again had an opposite effect (1959).

The purpose of this study is to determine the histological localization of tetracycline in the metastatic calcifications in the stomach wall induced by dihydrotachysterol, vitamin D_3 and parathyroid extract. The ordinary histological technique is supplemented by fluorescence microscopy and ultra-soft microradiography.

MATERIAL AND METHODS

Female albino rats aged 3 months and weighing about 150 g were used. During the experiment the animals were given ordinary laboratory food and water. The animals were divided into following groups:

Group 1. 8 animals. These were first given 10 mg of dihydrotachysterol (AT 10 Merck) through an oral tube and further 3 mg on the following day. The rats were given a single injection of tetracycline (Aureomycin intravenous, Lederle), 50 mg/kg body weight. Two of the rats were sacrificed on the 3rd, 5th, 8th and 10th days after treatment.

Group 2. (controls) 4 animals. These were given an injection of tetracycline as above. The animals were sacrificed at the same times as those in group 1, one at a time.

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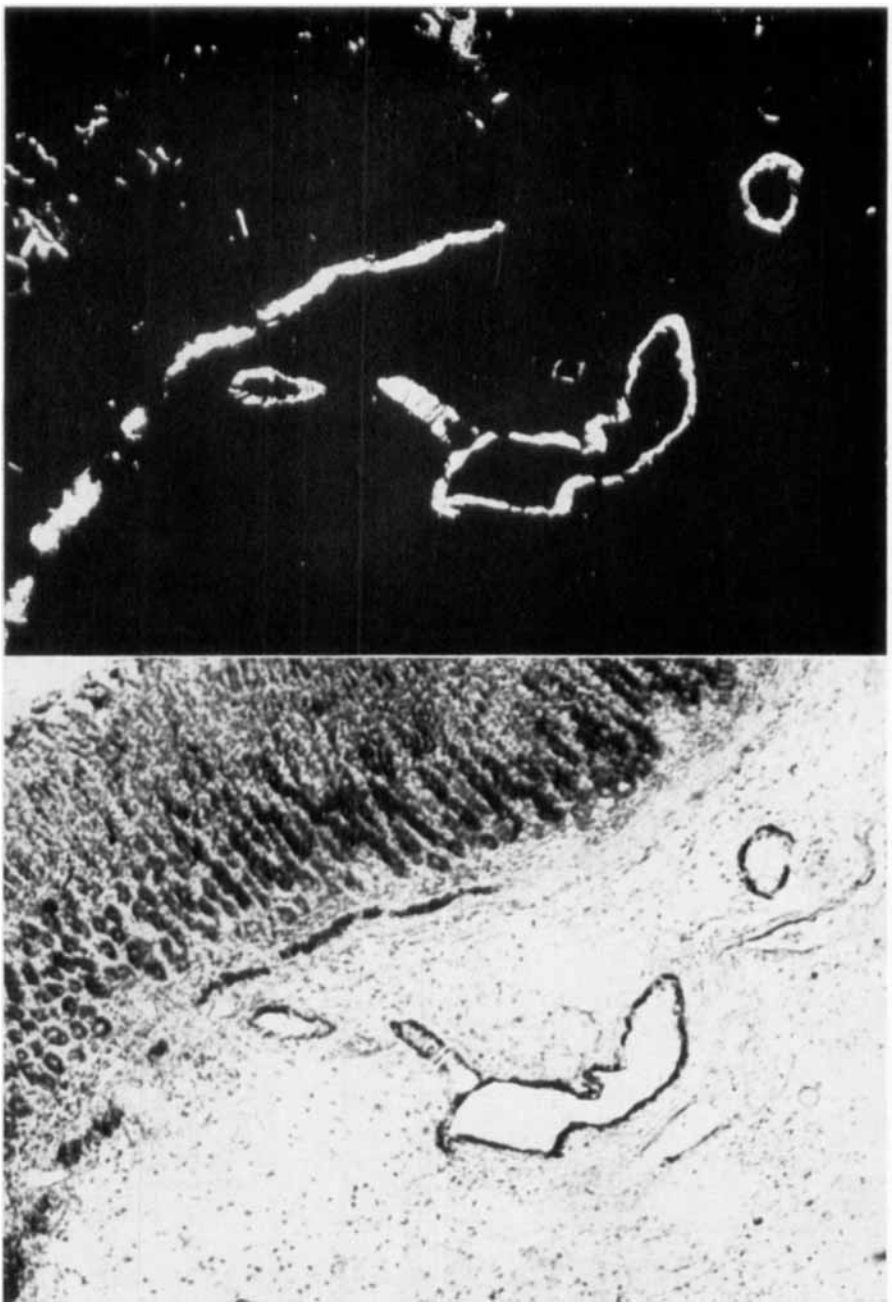

Figs. 1–2.

Fig. 1. A fluorescence microscopical view of a unstained frozen section. Magnification 200 ×.

Fig. 2. The same section as in Fig. 1 after staining with toluidine blue. Both figures show that the fluorescence is located in the vascular walls (middle and right), in the basal membrane and the middle portion of the mucosa. Magnification 200 ×.

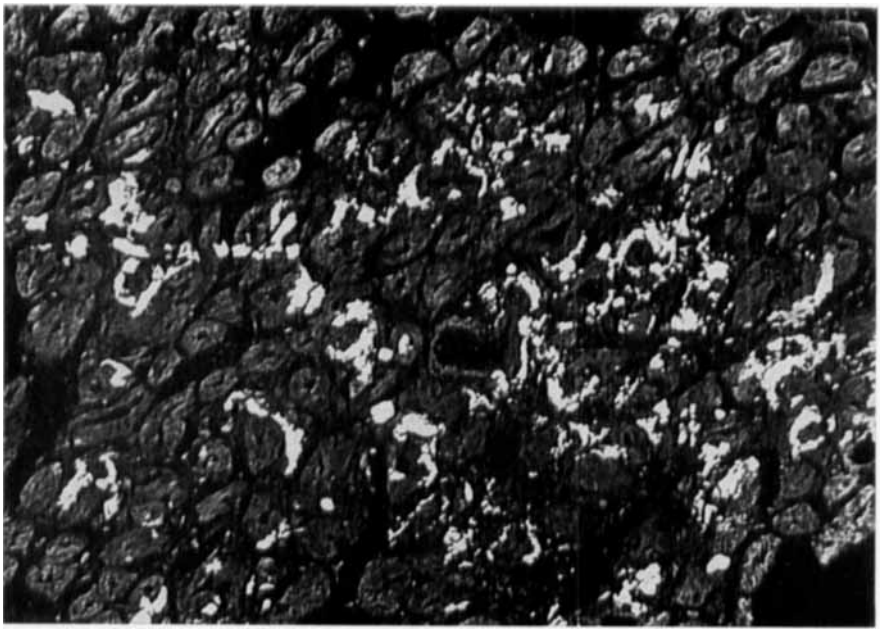

Fig. 3.
Microradiogram of 5 μ thick horizontal section from the midd-portion of mucosa. The glands are encircled by extracellular calcium salts. Exposed at 8–20 Å. Magnification 350 ×.

Group 3. 8 animals. These animals were treated exactly as animals in group 1, but AT 10 was replaced by 6 mg of vitamin D_3 (D_3-Vigantol, Bayer), which was given on each of the two days.

Group 4. (controls) 4 animals, which were treated as group 2 animals and used as control with group 3.

Two rats received an intramusclar injection of parathyroid extract (Para-Thor-Mone, Lilly). A dose of 50 IU was given for two days, and an injection of tetracycline like all animals in the series. These rats were sacrificed after 3 days.

When the animals were killed, the organs were examined in ultraviolet light (wawelength 3600 Å). At first frozen sections were made from fluoreescent areas of the stomach wall and studied by fluorescence microscopy and light microscopy after toluidine-blue staining. It was noted, however, that the ordinary technique for histological staining (formol fixation, alcohol-xylol, paraffin embedding) did not destroy the fluorescence and most sections could be treated in this way.

Fluorescence microscopy was used for unstained and deparafinized sections. Ultra-soft microradiography was used to study the calcium deposits at cellular level. Sections of 5 μ thickness were exposed to x-rays in the region of 8–20 Å (*Engström* 1956). After the sections had been studied by the methods mentioned above, they were stained with haematoxylin-eosin, haematoxylin-Van Gieson, periodic acid Schiff technique and mucicarmin.

RESULTS

When the organs were gross examined, it was observed that there was a strong yellow fluorescence in the stomach, lungs and kidneys of the animals treated with AT 10, vitamin D_3 or parthyroid extract, plus tetracycline. The control animals which had received only tetra-

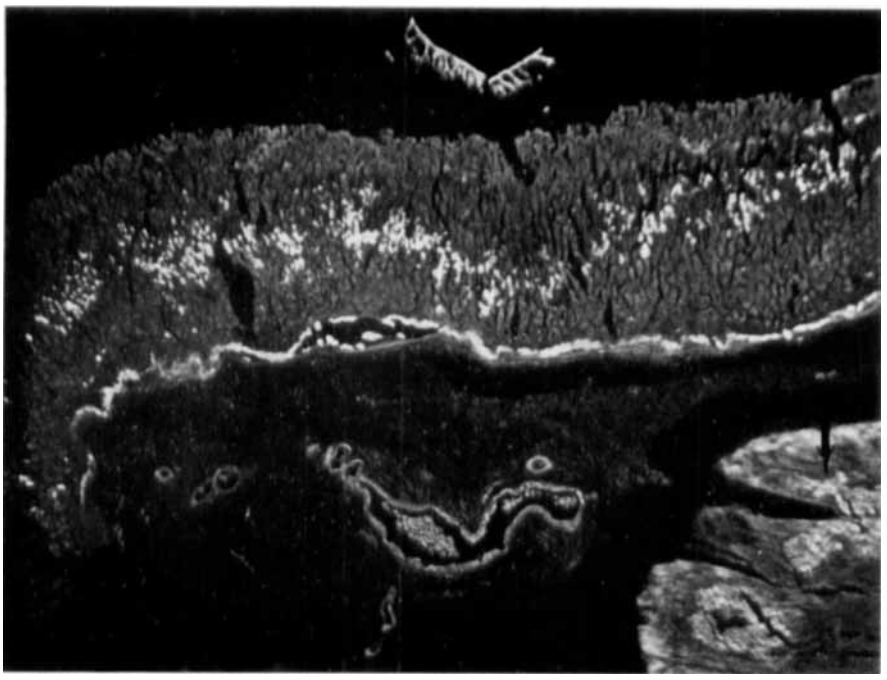

Fig. 4.
Microradiogramm of a 5 μ thick section from the gastric wall. Calcifications are present in the midd-portion of mucosa, in the basal membrane and in the muscular coat (arrow). Exposed at 8–20 Å. Magnification 100 ×.

cycline did not present any fluorescence in the organs mentioned. However, all the animals were noted to have a strong fluorescence in their bones.

Fluorescence microscopy showed that the fluorescence was typically located in the stomach wall. There was no difference in the groups treated with AT 10, vitamin D_3 or parathyroid extract. It is apparent that these three substances have an action of similar nature on the stomach. The results will be given in the following, considering only the length of the experiment.

Findings after 3 days: The fluorescence was observed in the basal membrane of the mucosa, in the walls of the blood vessels, in the muscular coat and a little in the middle portion of the mucosa (Figs. 1 and 2). Microscopy showed that these areas contained patches which were stained with haematoxylin. The intensity of staining reaction showed a strong variance. Microradiography showed that these pathological changes had a great density and were located extracellularly in the fibres of connective or muscular tissue (Fig. 3). The walls of the blood vessels did not show any abnormal density.

Findings after 5 days: The fluorescence had much increased and was located in the same areas mentioned above. The haematoxylin staining

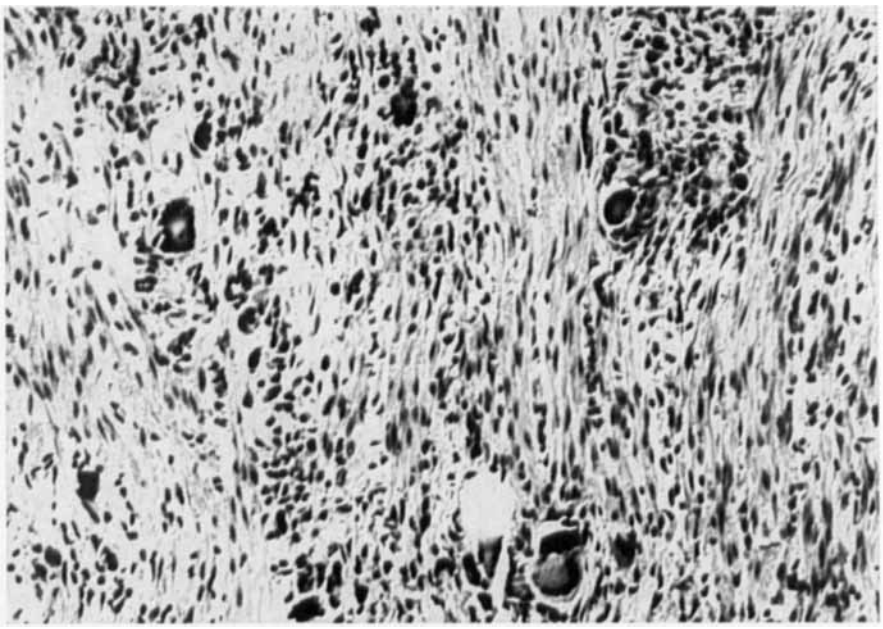

Fig. 5.
Multinucleated giant cells in a calcified focus of the muscular coat. 10 days after the treatment. Magnification 400 ×.

reaction had increased also. In the muscular tissue these areas were infiltrated with macrophages. Microradiography showed absorption variations in the same areas (Fig. 4).

Findings after 8 days: The fluorescence in the middle portion of mucosa had decreased, but remained unchanged in the other areas. Histological findings resembled the changes after 5 days. The macrophage infiltrations in the muscular tissue had increased, and there were some multinuclear giant cells also. Microradiography showed that the abnormal densities had remained unchanged except in the mucosa, were they had decreased.

Findings after 10 days: The fluorescence had diminished in the middle portion of mucosa, but remained unchanged elsewhere. The changes in the muscular tissue showed now plenty of multinucleated giant cells of foreign body type. Microradiography showed unchanged conditions.

DISCUSSION

It is certain that the abnormal densities, observed by micrography in the different parts of the stomach wall, consist of calcium salts. The methods used do not permit a more detailed analysis of their chemical structure.

The calcified areas were succesfully stained with haematoxylin, but

there was a great variation in the staining reaction. It could be observed that areas, showing a rather strong calcification by microradiography, stained very weakly with haematoxylin. This shows that haematoxylin staining is not a very reliable method for studying calcified structures. It is demonstrated that completely decalcified tuberculous foci still stain with haematoxylin (*Lindgren* 1961). It is apparent that haematoxylin does not stain calcium as such, but rather the matrix in which calcium salts are apt to deposit.

It was observed that tetracycline was localized in the metastatic calcifications. Chemically it has been shown that calcium and tetracycline produce a complex compound, which can be extracted from the tissues (*Kohn* 1961). Thus it is possible to use tetracycline as an intravital stain for calcium salts.

However, it was observed that tetracycline was located in the vascular wall, which never presented calcification. It is possible that this is due to the change in the ground substance caused by overdosage of parathyroid extract or related substances (*Engel* 1952, *Laskin et al.* 1956, *Gaillard* 1957). This change has apparently the ability of absorbing tetracycline also. The hyperfunction of the parathyroid gland or overdosage of related substances cause metastatic calcifications in different organs, especially in the orkans with high metabolic activity (*Lehr* 1956, *Selye* 1962). The localization has been explained by the assumption, that a rapid ionic exchange occurs in these organs which regulate the ionic balance of the organism. Possibilities for the deposition of calcium salts in these areas should be favourable (*Kleinman* 1928).

This study showed that the metastatic calcifications in the stomach wall were located extracellularly in the connective or muscular tissue fibres. The localization in the middle portion of gastric mucosa is interesting, and it is possible that this, in some way, is connected with the production of hydrochloric acid.

SUMMARY

The overdosage of dihydrotachysterol (AT 10), vitamine D_3 and parathyroid extract caused metastatic calcifications in the gastric wall of the albino rat. Tetracycline, given parenterally, fixated in these calcifications. This was shown by combined histological and fluorescence microscopical technique and by ultrasoft microradiograpsy. It is concluded that tetracycline can be used as an intravital staining for calcium salts. It is pointed out that tetracycline did also fixate in the changed ground substance of the vascular walls, which were not calcified. The metastatic calcifications were located extracellularly in the fibres of muscular and connective tissue.

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