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The Fluorescence of Tetracycline in Rats Treated with Dihydrotachysterol.

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Recently RALL et al. (1957) reported certain observations relating to the fluorescence of tetracyclines in the organism. They observed that tumour tissues from carcinoma patients exhibited a yellow fluorescence when irradiated with ultraviolet of wavelength 3,660 Å. The cause of this phenomenon was found to be tetracycline that had been administered to the patients. Similar phenomena were observed in mice into which various tumours had been implanted. The fluorescence persisted in the tumour tissues at least up to the 20th day after tetracycline had last been given. The fluorescence disappeared from normal tissues within 24 hours but was observed also in the bones during the 20 days the observations were continued.

MILCH et al. (1957) studied this fluorescence in the bones of all ordinary experimental animal species. The fluorescence in the bones and teeth did not diminish appreciably in intensity during 10 weeks after tetracycline had been administered. The fluorescent substance had concentrated in young animals in sites where bone formation was in progress in the epiphysis and especially in the endosteum and periosteum. Unossified cartilage did not fluoresce. The fluorescence was less intense in the older than in the younger animals. To explain the persistence of the fluorescence, these authors assumed that tetracycline combines with the bone matrix

to form a complex compound. It was considered possible that also calcium might participate in the formation of the complex.

Loo et al. (1957) studied the accumulation of fluorescent substance following administration of tetracycline to mice into which sarcoma had been implanted. They concluded that a weak complex is formed by tetracycline and some peptide and that this complex readily dissociates in an acid medium.

On the basis of these observations on the fluorescence produced by tetracycline in bones, the present writer has carried out an investigation on rats that had been first given fairly large doses of dihydrotachysterol and then tetracycline. The aim was to determine the effect of the metabolic changes effected in the bone and other organs on the development of fluorescence after administration of tetracycline.

Materials and Methods.

Twenty-four albino rats 2 months old were employed as experimental animals. Their mean weight was 190 g and before the experiments they had been given a free diet. No attention was paid to sex. One group of rats were fasted for two days. A group was given 10 mg of dihydrotachysterol (AT 10, E. Merck, Darmstadt) orally through a tube and a second 5 mg dose of AT 10 on the following day. All of the rats were given 10 mg of aureomycin (Aureomycin intravenous, Lederle) either intraperitoneally or intramuscularly on the second day. The rats that had fasted two days before the experiments were begun, were fed either a low calcium or a normal diet. The animals were killed 2—4 days after the tetracycline had been administered. The different organs were examined in ultraviolet light of 3,660 Å (UV light source: Die Analysen-Lampe, Original Hanau). Specimens were taken from the fluorescing regions of the kidneys, stomachs and heart muscles for microscopic examination. The specimens were preserved in formalin and stained with haematoxylin eosin. The distribution of the animals will be apparent from the accompanying table.

Results.

Two rats from each of groups 1 and 3 died on the second day after tetracycline had been administered. All the rats that were given AT 10 lost weight and their conditions deteriorated; this applied in particular to those that had fasted and were then kept on the low calcium diet. A distinct yellow fluorescence similar to that described by Milch et al. (1957) was noted in the bones

Table I.

Rats treated with dihydrotachysterol (AT 10) following injection of 10 mg aureomycin either i. p. or i. m. on the second day of experiment. The animals were killed 2—4 days after the tetracycline had been administered. The rats that had fasted two days before the experiment was begun were fed a calcium deficient diet under the experiment time.

Rat Groups	2 days fasting before exper.	Ca-deficient diet	Free diet	AT 10 10 mg	AT 10 5 mg	Killed days after tetra-cycl.
1. (4)[1]	+	+	—	+	+	4
2. (3)	—	—	—	+	—	2
3. (5)	—	—	—	—	—	4
4. (7)	+	+	—	—	—	4
5. (5)	—	—	+	—	—	4

[1] Numbers within brackets refer to the number of rats.

of the animals on exposure to ultraviolet light. No clear differences in the distribution and intensity of the fluorescence were observed between the different groups of animals. Group 4 that first fasted and then was kept on the low calcium diet, and group 5, the controls, did not exhibit fluorescence elsewhere than in the bones and teeth. A wide region with a yellow fluorescence containing necrotic tissue was observed in the muscle at the site of injection in those rats which were given tetracycline intramuscularly. No fluorescence was noted at the sites of peritoneal injection. All groups included both animals that had been given intramuscular injections and animals that had been given intraperitoneal injections.

All the animals that received AT 10 were found to have spotted yellow fluorescent regions on the surfaces of the kidneys. Also the deeper layers contained spotted fluorescent regions. In groups 1 and 3 the fluorescence in the kidneys was slightly more intense than in group 2. Extensive areas exhibiting a yellow fluorescence were observed in the corpus part walls of the stomach, but these were more clearly seen from the serosa than from the luminal side. Also this fluorescence was somewhat more intense in groups 1 and 3 than in group 2. A dotted fluorescence was noted both on the surface and in deeper layers of the heart muscle. This fluo-

rescence was weak in the animals of group 2. Small fluorescent spots were seen in the lungs and in the larger bronchi.

In the specimens which were taken from the fluorescent regions of the kidneys and stained with haematoxylin eosin, a blue mass could be distinguished in the media of the small arteries, blue granules in the epithelial cells of the tubules and dark blue regions, which could not be exactly defined as to their location, here and there in the vicinity of the tubules. No such stained areas were generally seen in the glomeruli. Small blue-coloured granules were observed at the roots of the gastric glands, and small deep blue areas sporadically in the muscle layers. Blue granular masses were observed here and there in the muscle cells of the heart which were in part necrotic.

The microscopic examination of fluorescing regions thus suggested beginning calcification of these organs. Similar changes have been effected with AT 10 in numerous earlier experiments (HOLTZ 1933, CARLSSON et al. 1955, SELYE 1957).

Discussion.

ALBRIGHT et al. 1938 and CARLSSON et al. 1955 have established that AT 10 and vitamin D do not differ essentially in their effect on the mineral metabolism if equivalent doses are given. Calcification of soft tissues may be effected with parathyreoid extracts and may occur in primary hyperparathyreosis in the same regions as it results after giving an excess of vitamin D (HUEPER 1927, WEIFE 1949, ALBRIGHT et al. 1948) and the changes observed cannot be distinguished histologically. The mechanism of action of these substances has not been definitely clarified. However, quite recently observations have been made that the parathyreoid hormone acts on the ground substance of the matrix associated with connective tissue, at least in the bone (ENGEL 1952, LASKIN et al. 1956, GAILLARD 1957), but possibly also elsewhere, *e. g.* in the kidneys (SHETLAR et al. 1956), and hence the primary change would take place in the ground substance and calcification would occur after the matrix has become depolymerized to a certain extent.

The mechanism of the action of vitamin D has been clarified in some degree (ELLIOT et al. 1956, DE LUCA et al. 1957) but the actual reason for the formation of calcified metastases in hyper-

vitaminosis is unknown. BAKER et al. (1954), have stated that renal calculi do not develop as a consequence of hypercalcemia alone.

Although the fluorescence resulting from administered tetracycline is observed in the bones which contain much calcium and in calcified metastases, the fluorescing compound does not necessarily have to be a complex formed by tetracycline and calcium. The present writer has performed partial hepatectomies in rats and observed a fluorescence due to tetracycline in the vicinity of the wounds as late as 20 days after the operation, but no calcification was noted on histological examination (in preparation). Ulcers effected in dogs also fluoresce after administration of tetracycline (in preparation).

One is tempted to associate the fluorescence due to tetracycline with the ground substance binding the connective tissue cells, which undergoes change when invaded by a malign tumour, in the vicinity of a wound, in inflamed and necrotic regions and in normal ossification (SHETLAR et al. 1949, SEIBERT et al. 1947, GERSH et al. 1949, CACHPOLE 1950, WEIMER et al. 1957). This view is supported by the observation of Loo et al. (1957), that tetracycline forms complex with some peptide.

Summary.

Typical calcified metastases were produced with AT 10 in the kidneys, stomachs, heart muscles and lungs of rats. Tetracycline given parenterally to these rats led to the appearance of regions in the calcified areas that fluoresced yellow in ultraviolet light. The intensity of the fluorescence was observed to increase in proportion to the dose of AT 10.

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