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Metastatic Calcification in Experimental Overdosage of Dihydrotachysterol in Rats

Effect of Tetracycline, DOCA and Cortisone

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Abstract

HÄKKINEN, I. P. T. Metastatic calcification in experimental overdosage of dihydrotachysterol in rats. Effect of tetracycline, DOCA and cortisone. *Acta physiol. scand.* 1959. *47.* 173—178. — Tetracycline and DOCA were observed to increase metastatic calcification in rats treated with dihydrotachysterol (AT_{10}). Cortisone had an opposite effect. DOCA increased tetracycline fluorescence in calcium metastatic regions, cortisone had again an opposite effect. So tetracycline fluorescense and metastatic calcification seemed to correlate. The way of fixation of tetracycline has been discussed.

Observations that Toluidin Blue increases metastatic calcification in overdosage of parathyreoid hormone in rats have been made (BAKER, REAVEN and SAWYER 1953, 1954). As the widely discussed ground substance (GERSH and CATCHPOLE 1949, SHETLAR *et al.* 1956, and others) depolymerizes under the effect of parathyreoid hormone, BAKER *et al.* (1954) assume that Toluidin Blue combines with chondroitin sulphate so that the ability of combining calcium also increases. On the other hand MILLER, WALDMAN and MC LEAN (1952)

Table I. Metastatic calcification in rats after AT_{10}. Tetracycline fluorescence in metastatic areas.

No. of animals	Treatment	The grade of fluorescence					
		Kidney					
		0		+		++	
		no.	%	no.	%	no.	%
7	AT_{10}+tetracycline	0	0	2	29	5	71
12	AT_{10}+tetracycline+cortisone	5	42	5	42	2	16
14	AT_{10}+tetracycline+DOCA	1	7	7	50	6	43
5	Tetracycline	5	100	0	0	0	0

have succeeded in preventing ossification of rachitic cartilage in rats with Toluidin Blue. BAKER *et al.* (1954) are not able to explain what causes this in a way opposite effect. BAKER *et al.* (1954) also studied the effect of DOCA on hyperparathyreoidism in rats and observed diminished calcification in the kidneys in 5 animals. This was explained by the phlogistic effect of DOCA, which would be the same as the increasing of polymerisation degree in ground substance. This conclusion sounds peculiar, as we know that mucopolysaccharides depolymerize in active connective tissue (GERSH *et al.* 1949). They further assumed that cortisone increases metastatic calcification. In some later works BACON, PATRICK and HOWSARD (1956) and LARON, MUHLETHALER and KLEIN (1958) observed that cortisone prevents calcification of kidneys in experimental hyperparathyreoidism. These investigations have mainly been undertaken to explain the function of parathyreoid hormone, but at the same time light has been thrown on the phlogistic-antiphlogistic effect of DOCA and cortisone, which (TURNER 1955, p. 197) seems evident. Some important qualities of the connective tissue have been examined in experimental conditions and it has been found that all three hormones, parathyreoid, DOCA and cortisone have an effect on it.

A further observation connected with the reaction of connective tissue (HÄKKINEN 1958) was the fixation of tetracycline in calcium metastatic regions produced by AT_{10} in rats. The further development of this observation led to the present work, where the aim is to explain the relation of fluorescing tetracycline in connective tissue in reactive state, and especially the effect of cortisone-DOCA on it.

Materials and Methods

Male albino rats, age about 7 months and mean weight 200 g, were employed as experimental animals. During the experiment the animals were given ordinary laboratory food and water. The animals were divided into following groups:

Effect of cortisone and DOCA on the fluorescence. See text.

The grade of fluorescence

Stomach						Heart muscle					
0		+		++		0		+		++	
no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
1	14	0	0	6	86	3	43	1	14	3	43
4	33	6	50	2	17	7	59	4	33	1	8
0	0	7	50	7	50	4	28	4	28	6	44
5	100	0	0	0	0	5	100	0	0	0	0

Group 1: 7 animals. These were given 10 mg of AT_{10} through an oral tube and 5 mg on the following day. The rats received a single injection of tetracycline i. m., 50 mg/kg body weight.

Group 2: 12 animals. These were given AT_{10} and tetracycline in the same way as group 1 and also 5 mg of cortisone acetate/day s. c.

Group 3: 14 animals. These were given AT_{10} and tetracycline in the same way as group 1, besides 1.5 mg DOCA/day i. m.

Group 4: 5 animals. AT_{10} in the same way as group 1.

Group 5: 5 animals. Tetracycline in the same way as group 1.

The injections of DOCA and cortisone were begun 2 days before feeding AT_{10} and they were continued for 7 days. AT_{10} was given in all 15 mg on 2 days and a single dose of tetracycline (Lederle Laboratory kindly presented the "Achromycin intravenous" used in the experiment), 4 days before the sacrification the dose being 50 mg/kg body weight. The rats were sacrified on the 8th day. The bones, kidneys, stomach and heart muscle were examined in UV-light. Histological specimens were taken from the yellow fluorescing regions of these organs. If there was found no fluorescence, a specimen was taken in the same way as from the fluorescing tissues. The specimens were fixed in formalin and stained with hematoxylin and eosin.

Results

In all other groups except that having received DOCA a decrease in weight was noted. The animals of the DOCA group either maintained their former weight or the weight increased slightly. This phenomenon may be explained by the retention of electrolytes and water. The weight in the group which received only tetracycline remained unaltered. The fluorescence caused by tetracycline was examined as previously reported (HÄKKINEN 1958). In corresponding histological specimens dark blue spots caused by calcium were examined. These contained necrosic parts and inflammatory cells in the heart muscle and in the connective tissue of the stomach. Dark blue spots were noted in the basal membrane of the cells in the kidneys and dark blue masses in the lumen of the tubulus was also found in more calcified tissues. BAKER *et al.*

Table II. Metastatic calcification in rats after AT_{10}. Effect of tetracycline, DOCA and cortisone

No of animals	Treatment	The grade of calcification					
		Kidney					
		0		+		++	
		no.	%	no.	%	no.	%
7	AT_{10}+tetracycline	1	14	6	86	0	0
12	AT_{10}+tetracycline+cortisone	3	25	9	75	0	0
14	AT_{10}+tetracycline+DOCA	1	7	10	71	3	22
5	AT_{10}	4	80	1	20	0	0
5	Tetracycline	5	100	0	0	0	0

(1954) have divided the calcified tissues into groups on the ground of this staining. The following grouping has been used in the tables: a negative result, slightly positive and strongly positive result. In Table I the different organs have been classified in reference to the fluorescence caused by tetracycline. 0 = no fluorescence, + = weak or only regional fluorescence, ++ = strong fluorescence.

The histological results have been classified in Table II. 0 = no dark blue colour caused by calcium. + = only in few places appearing colour. ++ = a strong dark blue colour in several places.

The difference between weak and strong fluorescence is not only the difference in intensity. The weak fluorescence means spotted slight yellow colour. The strong fluorescence is a compact colour.

The results show, that cortisone decreases the fluorescence of tetracycline and also calcification. The effect of DOCA is opposite and clear: fluorescence increases as well as calcification.

Comparing the group having received only AT_{10} with the group AT_{10}+ tetracycline there is noted more calcification in the latter than in the former. Comparing Table I and II in regard to the groups having received tetracycline it is found that the histological picture and tetracycline fluorescence correspond to each other rather well. The conclusion may be drawn that tetracycline fixes approximately in the same places as calcium and at the same time enhances calcification.

Discussion

On the basis of the works by RUBIN and HOWARD (1950), ENGEL (1952) BAKER *et al.* (1953, 1954) and SHETLAR *et al.* (1956) it may be considered evident that the parathyreoid gland has a direct effect on connective tissue and causes changes in the mucopolysaccharides of the ground substance. LASKIN and

on the calcification. See text.

The grade of calcification											
Stomach						Heart muscle					
0		+		++		0		+		++	
no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
3	43	4	57	0	0	2	29	3	42	2	29
5	42	7	58	0	0	7	58	4	34	1	8
1	7	11	79	2	14	1	7	7	50	6	43
4	80	0	0	1	20	0	0	4	80	1	20
5	100	0	0	0	0	5	100	0	0	0	0

ENGEL (1956) have studied the biochemistry of the bones more thoroughly in this connection and observed that the succinic dehydrogenase activity diminishes in overdosage of parathyreoid hormone. The oxygen consumption diminishes, glycogen decreases and the mucoproteins of the ground substance disaggregate. These effects are evidently on an intracellular level.

The mechanism has not been explained how calcification on the changed ground substance of soft tissues takes place. A corresponding calcification occurs in connection with the vitamin D and dihydrotachysterol (AT_{10}) overdosage (CARLSSON and LINDQUIST 1955, CRAWFORD *et al.* 1957), which has also been used in the present work.

Toluidin blue has before been used in experiments as a factor increasing metastatic calcification, (BAKER *et al.* 1953, 1954). SELYE (1957) has also used Phenylbutazone in the same way. The result of BAKER *et al.* in regard to DOCA is perhaps not right and they do not consider it reliable even themselves, because of the small material.

In the present work DOCA and tetracycline were observed to be factors increasing metastatic calcification in the overdosage of AT_{10}. Cortisone was observed to be a factor decreasing calcification. Because according to BAKER *et al.* (1954) Toluidin blue combines with chondroitin sulphate it would be tempting to think that tetracycline behaves in the same way. This assumption is perhaps supported by the observations of the present writer that after the hydrolysis the content of sulphate is greater in the tissues fluorescing with tetracycline than in their neighbouring healthy tissues (HÄKKINEN et HARTIALA 1959 and in preparation). These analyses have been carried out with experimental cincophen ulcers of dogs.

Heparin and Toluidin blue are antagonists in blood coagulation mechanism (ALLEN *et al.* 1949). It is interesting to see the relation of tetracycline and heparine to each others. It is perhaps appropriate to see the effect of tetracycline on ossified cartilage.

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