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THE EFFECT OF DIHYDROTACHYSTERIN (AT10) ON THE COLLAGEN MATURATION IN THE DENTIN OF RAT INCISORS[1] [2]

by

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The studies of previous investigators on the effect of the parathyroid hormone (*Irving et al.*, 1949; *Schour et al.*, 1934; *Schour & Ham*, 1934; *Schour et al.*, 1937; *Weinmann & Schour*, 1945) and D-vitamins (*Harris & Innes*, 1931; *Irving et al.*, 1949; *Schour & Ham*, 1934; *Schour et al.*, 1937; *Spreter v. Kreudenstein*, 1938; *Spreter v. Kreudenstein*, 1939; *Weinmann*, 1933) on dentin dealt only with the pattern of disturbances of mineralisation.

Kalnins and Ledina 1947 drew attention to the fact that hypercalcification of dentin in the experimental hyperparathyroidism is due to the malformation of matrix because of the inability of odontoblasts to produce collagen.

The task of the present work is to determine whether the hypercalcification of dentin provoked by dihydrotachysterin (AT10) depends on the disturbance of the maturation of collagen in the dentin, as it does with the parathyroid hormone.

MATERIAL AND METHODS

Irradiation with ultra-violet rays of ergosterin, dihydrocholesterin and their respective derivates results in the appearance of some substances such as toxisterin, vitamin D_2, tachysterin

[1] This work is a part of the still unpublished experiment on the "Effect of AT10 and the Parathyroid Hormone on Teeth and Bones".

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and dihydrotachysterin. With the exception of vitamin D_2, no effect or only an insignificant one is made by these substances on rickets; but they are similar to the parathyroid hormone in that they are all able to mobilise calcium in serum and show antitetanic effect. (*Gissel & Bufel,* 1942; *Holtz & Schreiber,* 1930; *Holtz et al.,* 1933/34; *Lenel* 1941/42). Therefore one of them, dihydrotachysterin, is also called "antitetanic preparation Nr. 10", or in short AT10. As does the parathyroid hormone AT10 also restores to normal conditions the disturbed calcification of dentin caused by experimental parathyroidectomy. (*Spreter v. Kreudenstein* 1938 and 1939).

According to Holtz *(Holtz & Schreiber* 1930; *Holtz et al.* 1933/34), 1.0 cc. of an oil solution of AT10 contains 20 "To Gr" units. ("To Gr" means "Toxisches Grenzdosis" of the author). 1 unit of "To Gr" is the minimum weight of a single dose of AT10, which is to be administered to an adult mouse for the purpose of reducing its body weight by 1/8 in 10 days. The preparations of the AT10 used in this experiment were manufactured in Bayer's or Wander's factories (Germany). The parathyroid hormone used was Parathyroid Extract EP6112, Lilly's (U.S.A.) "Para-Thor-Mone". 1.0 cc. of the solution of this preparation contains 20 Collip units. One Collip unit is equivalent to 5 U.S.P.

White rats placed on a diet consisting of bread and milk were divided into three groups (see Tables I, II and III). The first group received varying doses of AT10 daily. The second group of animals received parathyroid hormone and their dentinal changes were compared with those of the first group. All the animals if they did not die of their own accord were killed on the day after the last administration of the preparation. The untreated rats in the third group were used as controls. Immediately after the death of the animals the jaws were fixed in a 10 per cent formalin solution. Decalcification was performed in a 5 per cent solution of trichloracetic acid, and paraffin was used for embedding. The jaws containing the upper incisors and part of the alveolar bone containing the lower incisors were examined by means of longitudinal sections. The molar area of mandibles was also sectioned in sagittal plane.

The following staining methods for estimating the collagen

Table I

Experimental Data on Rats receiving Dihydrotachysterin (AT10)

No. of Rats	Body Weight in GM at the Begin. of Experiment	Daily Dosage of AT10 in cc. per Animal	Method of application of AT10	Duration of Experiment in Days	Final Body Weight in GM	Kind of Death
R4	105	0.3 0.4 1.1 0.0 0.4 0.4 0.4 — — — — —	per os	7	102	Killed
467/1	143	0.4 0.5 0.7 0.8 0.8 0.8 0.8 0.8 — — — —	subcutan	8	131	spontaneously
467/2	81	0.4 0.5 0.7 0.8 0.8 0.8 0.8 0.8 — — — —	»	8	70	»
467/3	128	0.4 0.5 0.7 0.8 0.8 0.8 0.8 0.8 — — — —	»	8	119	»
100/1	110	1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	stomacally	12	85	killed
100/2	117	1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	»	12	86	»
100/3	99	1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 —	»	11	63	spontaneously
100/4	93	1.0 1.0 1.0 1.0 1.0 1.0 — — — — — —	»	6	81	killed
100/5	95	1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 — —	»	10	67	»
100/6	125	1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 — —	—	10	76	»

Table II

Experimental Data on Rats receiving Parathyroid Hormone

No. of Rats	Body Weight in GM at the Begin. of Experiment	Daily Dosage of Parathyroid Hormone in cc. per Animal								Method of Application of Parathyroid Hormone	Duration of Experiment in Days	Final Body Weight in GM	Kind of Death
R1	92	1.5	1.5	3.0	0.0	1.5	1.5	1.5	—	Subcutan	7	98	Killed
225/2	170	2.5	2.5	2.5	2.5	2.5	—	—	—	»	5	159	Spontaneously
463/1	99	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	»	8	120	Killed
463/2	89	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	»	8	108	»
463/3	95	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	»	8	109	»
463/4	123	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	»	8	134	»

start in the tissues were employed. Firstly Hansen's staining method with iron trioxyhematin and the following counter-staining with acid fuchsin and picric acid. In this manner the collagen of dentin in the treated slides is stained red. Azan staining represents the Mallory method modified by Heidenhain. Here the collagen of dentin appears in blue. The disturbance of collagen maturation in dentin appears in the form of colourless stripes in the slides stained according to these two methods (Figs. 2, 4, 6, 10, 11, 12, 13). The staining of the calcium content of the bones and of the dentin was performed by means of Bock's method.

RESULTS

In the control animals the rate of body weight showed an increase (Table III) and their incisors exhibited both normal mineralisation and normal maturation of the collagen in dentin. On the contrary, in all the groups of rats treated with AT10 the rate of body weight showed a stoppage or decrease (Table I). The long bones were more fragile (determined by manual fracturing) than those in the controls.

Table III

Experimental Data on Control Rats

No. of Rats	Body Weight in GM at the Begin. of Experiment	Duration of Experiment in Days	Final Body Weight in GM	Kind of Death
80/3	126	12	164	Killed
80/6	61	12	68	»
460/1	90	9	110	»
460/2	114	6	120	»
460/3	101	9	120	»

The histological examination shows that the feature of "calciotraumatic response" in the dentin is generally in accord with results previously described by other investigators concerning disturbances of mineral metabolism caused by the administration of the parathyroid hormone and D-vitamins; this "calciotraumatic response" always appears in labial dentin (where the

portion of the incisor is covered with mature enamel) and independently of the duration of the experiment. Bock's staining reveals that the dentin developed during the first period of the effect of treatment with AT10 or parathormone remained uncalcified (Fig. 1). The dentin layer formed later is hypercalcified and is about 2—3 times wider than the uncalcified layer. On pulpal side this hypercalcified layer is covered with predentin of normal width. Hansen's and azan stainings show that the uncalcified layer mentioned could be subdivided into an older and a younger zone (Fig. 2). The older zone consists of a matured collagen, which is pulpally followed by an insufficiently collagenised or collagenless layer. The younger hypercalcified layer reveals reduced or normal maturation of collagen.

These features reveal a close relation between the disturbance of collagen maturation and the hypercalcification in dentin. It is obvious that the odontoblasts first lose their ability to produce mineralisation, and predentin forming at that time (the beginning of the response to the effect of AT10) remain uncalcified. The

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Fig. 1. Hypercalcification of dentin owing to effect of AT10. X, calciotraumatic line, demarcating the normal dentin, which developed before the response of odontoblasts to the action of AT10. a and b, dentin portions, formed under the effect of AT10. a, older dentin zone which failed to calcify. b, younger dentin zone, which is hypercalcified and pulpally lined by normally formed predentin. Labial dentin of the middle third of incisor. (Rat Nr. 100/6, Bock. 450×).

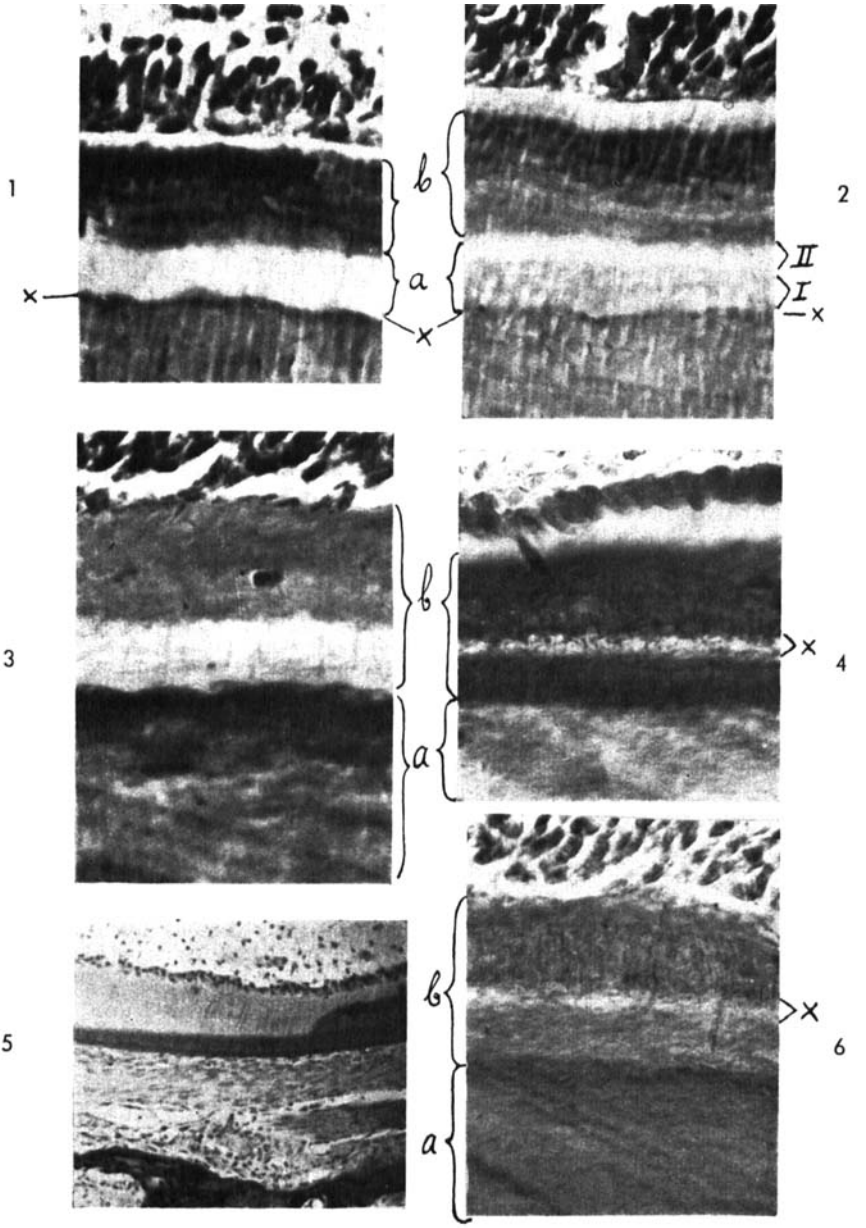
Fig. 2. The same specimen as shown in Fig. 1 but stained according to Hansen. X, calciotraumatic line. a, older zone which is hypocalcified and consists of two layers; I, with normal maturation of collagen and II, the layer where the deposition of collagen failed. b, younger zone, which shows that the production of collagen is resumed. (500×).

Fig. 3. Delay of calcification in lingual dentin of apical third of incisor owing to AT10 effect. a, older, well calcified zone formed before the response of odontoblasts to AT10. b, younger wide hypocalcified zone which developed during the AT10 action. (Rat Nr. 100/5, Bock. 500×).

Fig. 4. The effect of AT10 upon the collagen maturation in the lingual dentin. The same specimen as shown in Fig. 3 but stained with azan. a, older dentin portion (light grey in the picture) formed before the effect of AT10. b, younger zone (dark grey in the picture), which corresponds to the hypocalcified zone of Fig. 3. This zone shows disturbance of collagen maturation due to AT10 action in the form of a colourless stripe, x (450×).

Fig. 5. Delay of calcification owing to AT10 action. Lingual dentin of apical third of incisor. (Rat Nr. 467/3, Bock. 44×).

Fig. 6. The effect of AT10 on collagen maturation in the lingual dentin. The same specimen as in Figs. 3 and 4 but stained according to Hansen. a, older zone. b, younger zone with collagenless stripe, x (500×).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production of collagen during this phase is not affected. The second stage of the reaction of odontoblasts to AT10 appears in the complete or partial stoppage or reduction of collagen production. If during this phase odontoblasts later resume their ability to lay down mineral salts, hypercalcification of dentin matrix takes place, which in its pattern suggests the appearance of dentinsclerosis (Fig. 13).

Owing to the prolonged duration of AT10 action, the corresponding phases of collagen malformation and hypercalcification can be repeated and then two or more bands of interference in maturation can be observed (Fig. 11). The relationship between collagen malformation and the hypercalcification of dentin is the same in rats treated with AT10 or with parathyroid hormone (Figs. 9 and 12). The disturbances of collagen maturation could also be seen in lingual dentin in the apical portion of the tooth (Figs. 3, 4, 6), where the action of AT10 provoked instead of dentin hypercalcification, delay in mineralisation (Fig. 5) or the irregular development of dentin (Fig. 7). Examination of the alveolar bone reveals that osteoblasts react to AT10 in about the

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Fig. 7. The irregular formation of the labial dentin owing to AT10 action. Apical third of incisor. (Rat Nr. 100/4, Htx-eos, 44×).

Fig. 8. The effect of AT10 on alveolar bone of mandible. Alveolar process is porotic and shows amorph calcification of osteogenetic zone. (Rat Nr. 102/2, Bock. 44×).

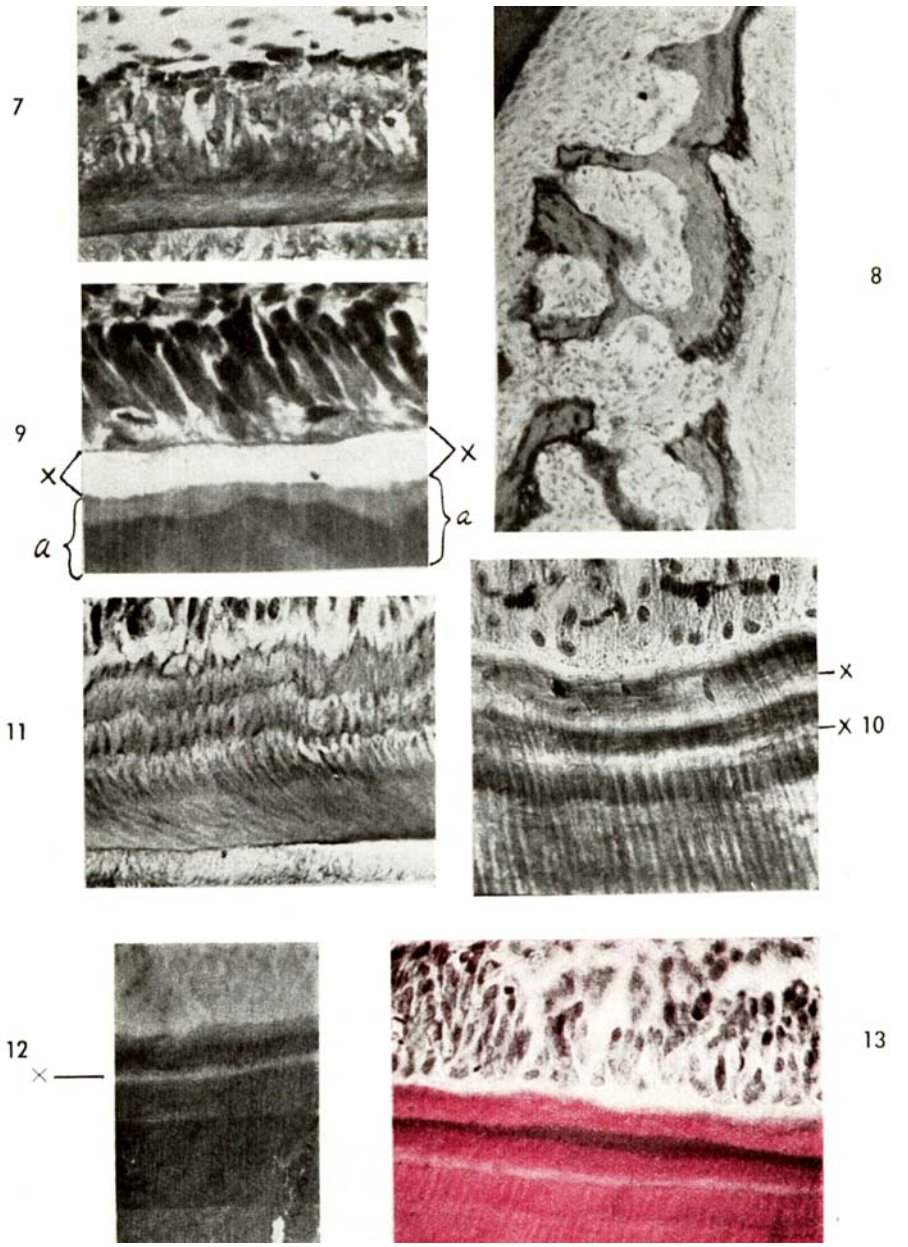
Fig. 9. Malformation of the dentin matrix due to effect of parathyroid hormone. a, normally developed dentin portion. x, uncalcified and collagenless dentin matrix. Labial dentin of the middle portion of incisor. (Rat Nr. 463/2, Bock. 500×).

Fig. 10. Striation of dentin owing to malformation of collagen in latent scurvy in molar of guinea pig. 49 days scorbutogenic diet with supplement of 1 mg. of ascorbic acid (1.0 c.c. of 0.1 per cent solution) by means of subcutaneous injections every second day. (Hansen, 150×).

Fig. 11. Striation of dentin due to repeated malformation of collagen in the incisor of rat receiving AT10. x, collagenless stripes. Labial dentin of the middle third of the tooth. (Animal Nr. 100/1, azan, 160×).

Fig. 12. Malformation of collagen in incisor of rat receiving parathyroid hormone. X—. Collagenless stripe in dentin which developed owing to hormone action. Labial dentin of the middle third of incisor. The colored picture of this slide is presented as Fig. 19 in the work of Kalnius & Ledina 1947. (Rt. Nr. 225/2, Hansen, 160×).

Fig. 13. Malformation of collagen and hypercalcification of the collagenless zone owing to AT10 action. Dentin with normal content of collagen is red. Collagenless stripes is white and that one hypercalcified is blue. Labial dentin of middle third of incisor. (Rt Nr. 100/1, Hansen, 160×).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same manner as odontoblasts. This results in the appearance of a hypercalcified seam instead of osteoid on the side of tension of periodontal fibres, where the apposition of new bone takes place (Fig. 8). The insufficient maturation of collagen is one of the factors causing the fragility of long bones observed in the dissection.

DISCUSSION

The present work shows that diohydrotachysterin (AT10) provoke the disturbance of collagen maturation with subsequent hypercalcification of dentin. This is in accordance with similar findings of *Kalnins* and *Ledina* 1947 in their studies on the effect of parathyroid hormone on dentin. It is also to some extent the case with the experiments of *Irving* and *Weinmann* 1948, who concluded that under the influence of strontium in predentin, which forms immediately after the injection, the differentiation of fibrils is disturbed, with a result that there is an excessive amount of calcioreceptive cementing substance, this portion therefore becoming hypercalcified.

It was presumed by other workers that dentinal changes in calcification, owing to the effect of parathyroid hormone (*Schour & Ham* 1934) or florine (*Irving* 1943, *Irving & Nienaber* 1946, *Irving* 1946) are in close relation to the rise and fall of the serum calcium level. The change of the calcium metabolism in the body as a whole is primary, and the odontoblastic damage, secondary (*Irving & Weinmann* 1948), *Irving* 1943, *Irving & Nienaber* 1946 and *Irving* 1946). Whether this is true or not can be seen from the results of the previous experiment on the effect of the parathyroid hormone on dentin of scorbutic guinea pigs (*Kalnins & Ledina* 1947). It was found in latent scurvy that, due to scorbutic disfunction of odontoblasts, the dentin hypercalcification and striation (caused by parathyroid hormone) appears more apically than in normal animals. On the other hand, in manifest scurvy the parathyroid hormone had no effect on dentin structure, because scorbutically degenerated odontoblasts had lost their ability to react.

Finally it is possible to produce dentin striation in a specially arranged experiment with latent scurvy only (*Kalnins* 1953), in

which hypercalcification of dentin depends on the malformation of dentin matrix (Fig. 10). Since in scurvy the calcium metabolism of the body is not affected, it is evident that the appearance of dentin striation here is due to the disturbance of function of odontoblasts only.

SUMMARY AND CONCLUSION

(1) The effect of Dihydrotachysterin (AT10) on the dentin was examined in the incisors of 10 rats, and the results were compared with those in the dentin of the rats receiving parathyroid hormone (6 rats).

(2) It was found that as in the experimental hyperparathyroidism, also during the action of AT10 the disturbance of collagen maturation precedes the hypercalcification of dentin.

(3) The role of odontoblasts in the appearance of dentin hypercalcification has been discussed.

The main part of the experiment was performed at The Pharmaceutical Laboratory, Stockholm, Sweden. The author wishes to express to the Director of the Laboratory Professor H. Rydin, M.D., his most sincere appreciation for the help given and the interest shown in this work.

ZUSAMMENFASSUNG

DIE WIRKUNG DES DIHYDROTACHYSTERINS (AT10) AUF DIE KOLLAGENREIFEN IM DENTIN DES RATTENSCHNEIDEZAHNES

1) Die Wirkung des Dihydrotachysterins (AT10) auf das Dentin des Schneidezahnes der 10 Ratten wurde untersucht und das Resultat mit Dentinveränderungen an Schneidezähnen der Ratten (6 Tiere), die das Epithelkörperchenhormon erhielten, verglichen.

2) Es wurde dasselbe festgestellt wie bei den Versuchen mit der experimentellen Hyperparathyroidismus, nämlich, dass auch infolge der Wirkung des AT10 die Störung der Kollagenreifen der Überverkalkung des Dentin vorausgeht.

3) Die Rolle der Odontoblasten im Vorgang der Dentinüberverkalkung wurde besprochen.

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