From the Medico-Chemical Laboratory and the Odontological Institute of Helsinki University.

The Incisor Teeth of Guinea-pigs in Vitamin-A Deficiency.

By

MAURI POHTO,

Helsinki.

616.31402 : 612.392.01
612.392.01 : 616.314

In a previous work I have given a detailed account of the changes in the continually growing incisors of rats caused by a diet deficient in vitamin A. In connection with that work I also carried out experiments on guinea-pigs, the results of which are given in this article.

Hitherto only WOLBACH and HOWE (1928, 1933) have studied these changes in the incisors of guinea-pigs. From their investigations they conclude that the most important of these changes is the atrophy of the enamel organ; the ameloblasts and their basal cells undergo granular degeneration and disintegrate; but before the complete disappearance of the enamel organ, calcified globules appear between the atrophying cells of this organ. They state that atrophy of the odontoblasts takes place later, appearing first on the lingual, later also on the lateral sides of the tooth. In these places processes form from the dentine towards the pulp centre, and then calcify. In these processes no dentine canals are found; they are surrounded by osteoblasts some of which become imprisoned in them. The cementum is thickened in some places.

In addition to the experiments on guinea-pigs described above, WOLBACH and HOWE (1925, 1933) have also, like myself, carried out corresponding experiments on rats. In the work mentioned above I showed that the results of their investigations differ greatly from mine. For that reason it will be of interest to compare their results with mine to see whether differences again occur, perhaps of a corresponding kind.

Material.

My material, kindly placed at my disposal by the Head of the Medico-Chemical Laboratory Prof. Dr. P. E. SIMOLA, comprises 11 guinea-pigs fed on a diet deficient in vitamin A and 5 control animals.

The 11 guinea-pigs received the following diet, used by SIMOLA (1932) for experiments with vitamins A and C:

Oats	80 parts
Wheat bran	80 »
Casein	8 »
$CaCo_3$	8 »
NaCl	2 »

The demand for vitamin C was satisfied by giving the guinea-pigs with a pipette 5 ccm of orange juice daily. The demand for vitamin D was satisfied with Vigantol diluted with arachid oil.

The control animals were given in addition to this food vitamin A in the form of Vogan diluted with arachid oil.

The weights of the animals when the diet started, the duration of this diet, and the weights at the end of the experiments are shown in the following table.

No. of guinea-pig	Weight when diet started	Deficiency period	Final weight
27	170 g	29 days	185 g
19	160 »	31 »	213 »
25	160 »	31 »	257 »
22	180 »	33 »	185 »
18	170 »	36 »	232 »
23	145 »	36 »	191 »
12	190 »	37 »	245 »
24	155 »	38 »	197 »
13	185 »	39 »	245 »
14	210 »	39 »	230 »
21	160 »	41 »	180 »

The incisors of the guinea-pigs were fixed in 10 % Formalin and microscopical specimens were prepared using the paraffin method. Delafield's Hematoxylin-Eosin was used as a general stain.

To describe more exactly the changes in the incisors of guinea-pigs, I have divided the tooth into three parts, i. e. the basal end,

the middle part, and the incisal end. The basal end is that part of the incisor near the first molar in the lower jaw; the middle part is surrounded by the alveolus, and the incisal part is the free point of the tooth. Fig. 1.

Results.

In the microscopical examination of the incisors of guinea-pigs fed on a diet deficient in vitamin A, it is observed that great changes take place during deficiency.

Of these changes atrophy of the odontoblasts is the prevailing symptom of deficiency. This is observed in the incisors of all the guinea-pigs all over the tooth except at the extreme base.

Atrophy of odontoblasts leads to a decrease in the size of cells, the regular row of cells becomes irregular as the connection with the dentine is broken, and finally the cells disappear completely.

Atrophy of odontoblasts appears near the base only on the lingual side. Proceeding towards the point it involves also the lateral sides, and even the lingual side from about the middle of the tooth to the incisal end.

Changes in connection with the atrophy of odontoblasts are also observed in the teeth of all the guinea-pigs. Of these changes the deposition of calcifying basic substance on the dead dentine is very conspicuous. It takes place first at the base of the middle part as small processes from the dentine wall towards the centre of the pulp on the lingual and lateral sides. These processes increase in size towards the incisal end, they cover the dentine which has died owing to atrophy of the odontoblasts, and finally fill the greater part of the pulp cavity. Now they can be observed also on the labial side of the tooth, though to a less extent than elsewhere. Fig. 2.

The odontoblasts adjoining the processes form at first a more or less irregular line, rapidly atrophy, and finally disappear completely. Connective tissue cells of the pulp, though not in large numbers, collect round the processes after the disappearance of odontoblasts. They sometimes form groups resembling odontoblasts in shape and arrangement. As the processes grow, atrophying odonto- and connective tissue cells of the pulp become imprisoned in them. Fig. 3.

These processes are a matrix resembling predentine. They atrophy to some extent during growth but can be clearly distin-

guished from the dentine owing to their considerably lighter stain. The boundary between the processes and the dentine is more clearly discernible the nearer they are to the incisal end. The silver stain specimens show that the processes have formed round the v. Korff's fibres which have become swollen owing to the deposition of basic substance. The connective tissue cells of the pulp appear to be responsible for this deposition. Fig. 5.

On the lingual side of the tooth all other guinea-pigs except numbers 27, 19 and 25 show hard tissue formation on the pulp side of the odontoblasts; it begins at the basal end and proceeds towards the point. Guinea-pigs 27, 19 and 25 in the corresponding areas show dentine processes of the kind described above.

In the middle part, especially towards the incisal end, hard tissue sometimes forms elsewhere than on the lingual side.

The hard tissue arises from the deposition of a matrix resembling predentine in the immediate neighbourhood of the odontoblasts on the part adjoining the pulp. The calcification is here greater than in the processes mentioned above. The odontoblasts which are left between the dentine and the hard tissue atrophy rapidly and finally disappear completely, so that the hard tissue now borders directly on the dentine. Figs. 6, 7, and 8.

The connective tissue cells of the pulp are responsible for the development of the hard tissue; these cells arrange themselves in a regular row and acquire the long shape of odontoblasts. As deficiency continues, however, they atrophy and now processes from the hard tissue begin to push forward towards the centre of the pulp in the way described above. Fig. 8.

Changes in the enamel organ of the teeth of all the guinea-pigs have occurred during the deficiency diet. Atrophy appears in the ameloblasts and their basal cells, but only at the base of the tooth. The atrophy has led to a breaking up of the regular arrangement of these cells, some of which on being set loose disappear completely. No changes are observed in the enamel layer. Figs. 9 and 10.

Examination of Results.

The table given above shows that the deficiency periods of the guinea-pigs do not greatly vary. For this reason it is not possible to follow the changes due to vitamin A deficiency as closely as it was in my experiments on rats. My material, however, in my

opinion gives a clear picture of the guinea-pig's incisor during vitamin A deficiency and some indications of the progress of this deficiency in the tooth.

It is to be noted that the deficiency pictures of the teeth of all my guinea-pigs are rather similar. The lack of greater variations is due to the slight difference in weight and in the diet periods, but it is clear that the results could not have differed considerably even with a larger number of guinea-pigs.

It is noticeable that the atrophy of the enamel organ is limited to the base of the tooth and that no changes whatever occur in the enamel. In my opinion these circumstances show that atrophy of the enamel organ does not appear at an early stage of deficiency because it would have extended over a larger area and have had a disturbing effect on the formation of enamel. On the other hand, atrophy of the odontoblasts can be observed over the whole length of tooth except at the extreme base. The great changes in the structure of the tooth wall have proceeded so far in the teeth that the initial cause, atrophy of the odontoblasts, must have been active for a long time. Thus it is in my opinion obvious that atrophy of odontoblasts with its consequences appears in the teeth of guinea-pigs at an earlier stage of the deficiency diet than atrophy of the enamel organ.

At an early stage the dentine processes are bordered by atrophying odontoblasts, the irregular function of which is the initial cause of these processes. Later, when atrophy causes the odontoblasts to disappear completely, pulp cells collect round the processes, though not in large numbers. The action of these pulp cells presumably causes an intense growth of the processes.

Hard tissue formation on the pulp side of the odontoblasts is observed without any preceding atrophy of the odontoblasts or deposition of hard tissue formation over them. As in the case of the processes, this is presumably due to the function of pulp cells in the region of the odontoblasts, of which the action is irregular.

On the basis of the above, it is in my opinion obvious that atrophy of odontoblasts is the first symptom of deficiency, which causes discernible changes in the teeth of the guinea-pigs. It can even be said that atrophy of the odontoblasts and the changes due to it are positively characteristic of the histological picture of the incisors of my experimental guinea-pigs.

A comparison between the results obtained by WOLBACH and HOWE, who have previously studied this question, and mine shows

considerable differences. These writers stated that atrophy of the enamel organ is the first deficiency symptom, and is associated with disturbances in the formation of enamel. For my own part, I have come to the conclusion that atrophy of the enamel organ appears only at a later stage of deficiency, and I have not observed any changes in the structure of the enamel.

WOLBACH'S and HOWE'S results regarding the atrophy of odontoblasts differ from mine in that they consider the atrophy to be limited to the lingual and lateral sides, while I have always observed it also on the labial side of the tooth, though to a less extent than elsewhere.

These research workers have found processes from the dentine wall, but not on the labial side of the tooth. I have observed them there also. They have also noticed a thickening of the cementum, which I have not been able to find.

When describing WOLBACH'S and HOWE'S and my own experiments on rats I particularly noted that our observations differed of the changes in the enamel organ; this difference was still greater than with the guinea-pigs, but of a corresponding kind. At that time I considered this difference as due to their excluding vitamin D as well as vitamin A from the diet of their rats. The same cause may be responsible for the difference in the results of our experiments on guinea-pigs; they have excluded vitamin D also from the diet of their guinea-pigs.

The question of the effect of vitamin A on the development of the tooth is, however, very wide and the examination of available points of comparison would take me too far from the real subject of this article.

Summary.

My material, comprising 11 guinea-pigs fed on vitamin-A-free diet and 5 control animals, has been studied with regard to changes caused in the incisors by vitamin A deficiency. The results were as follows:

The most important effect of vitamin A deficiency is atrophy of the odontoblasts; the atrophying odontoblasts cause the formation of processes from the dentine wall towards the pulp centre. The connective tissue cells of the pulp appear to be responsible for the continued growth of these processes. They finally fill the greater part of the pulp cavity. They are formed of a matrix resembling predentine, which calcifies.

On the pulp side of the odontoblasts hard tissue forms in some places. The odontoblasts between it and the dentine atrophy rapidly and finally disappear completely. The hard tissue is a matrix which resembles predentine and calcifies. The deposition of the hard tissue is caused by connective tissue cells of the pulp, which resemble odontoblasts in shape.

Atrophy of the enamel organ appears only in a limited area at the base of the tooth. The ameloblasts and their basal cells atrophy and some of them disappear completely. No changes are observed in the enamel layer.

The atrophy of the odontoblasts and its consequences appear to be the prevailing change caused in the incisors of guinea-pigs by vitamin A deficiency.

Zusammenfassung.

Mein Material, das 11 Meerschweinchen, die auf A-vitaminlose Kost gestellt worden waren, und 5 Kontrolltiere umfasst, wurde in Bezug auf die Veränderungen studiert, die durch A-Vitaminmangel an den Schneidezähnen verursacht waren. Nachstehend die Untersuchungsergebnisse.

Die hauptsächlichste Wirkung des A-Vitamin-Mangels ist Atrophie der Odontoblasten; die atrophierenden Odontoblasten verursachen die Bildung von Auswüchsen der Dentinwand zum Pulpazentrum hin. Die Bindegewebszellen der Pulpa scheinen das weitere Wachstum dieser Auswüchse zu verursachen und füllen schliesslich die Pulpahöhle aus. Sie sind aus einer dem Prädentin gleichenden Matrix gebildet, die verkalkt.

Auf der Pulpaseite der Odontoblasten bildet sich an manchen Stellen Hartgewebe. Die Odontoblasten zwischen diesen und dem Dentin atrophieren rasch und verschwinden schliesslich vollständig. Das Hartgewebe ist eine dem Prädentin gleichende Matrix, die verkalkt. Die Deposition dieses Hartgewebes ist durch Bindegewebszellen der Pulpa verursacht, die in ihrer Form den Odontoblasten ähneln.

Atrophie des Schmelzorgans zeigt sich nur auf einem beschränkten Gebiet an der Zahnbasis. Die Ameloblasten und ihre Basalzellen atrophieren und einige von ihnen verschwinden vollständig. In der Schmelzschicht sind keine Veränderungen zu beobachten.

Die Atrophie der Odontoblasten und die Folgen dieser Atrophie scheinen die vorherrschende Veränderung zu sein, die durch A-Vitamin-Mangel an den Schneidezähnen von Meerschweinchen verursacht wurden.

Résumé.

Mes observations portant sur 11 cobayes privés de vitamine A, ainsi que sur cinq bêtes de contrôle, furent destinées à l'étude des modifications des incisives, résultant de l'absence de vitamine A. Voir ci-après les résultats d'expérience.

L'effet principal du manque de vitamine A est une atrophie des odontoblastes; les odontoblastes atrophiés ont pour effet de créer des excroissances de la paroi de dentine en direction de la cavité pulpaire. Les cellules conjonctives semblent prendre la suite de ce développement et finissent par obstruer complètement la cavité pulpaire. Ces excroissances contiennent une substance, dont la nature rappelle celle de la couche génératrice, la calcification en plus.

Dans la portion tournée vers la pulpe des odontoblastes se dépose en plusieurs endroits un tissu solide. Les odontoblastes situés entre ce dernier et la dentine dégénèrent rapidement et finissent par disparaître complètement. Ce tissu solide rapelle, la calcification en plus, la pro-dentine de la couche-mère. Sa déposition semble due aux cellules conjonctives de la pulpe, qui ressemblent par leur forme aux odontoblastes.

L'atrophie de la fonction dissolvante n'apparait qu'en certaines régions bien délimitées de la base dentaire. Les éléments générateurs d'émail et leurs cellules de soutien s'atrophient complètement et certaines d'entre elles viennent à disparaitre sans laisser de trace. Aucune modification à signaler en ce qui concerne la couche dissolvante.

L'atrophie des odontoblastes avec les effets que cette atrophie entraine paraissent être les altérations prédominantes, concernant les incisives des cobayes, attribuées au manque de vitamine A.

References.

POHTO, MAURI: Suomen Hammasl. Seuran toim. LX, 1, 1932.
SIMOLA, P. E.: Acta Duodecim, Ser. A, XVI, 1932.
WOLBACH, S. B., & HOWE, PERCY R.: A. of Path. 5, 1928. Am. J. of Path. *9*, 3, 1933.

Adress: Museokatu 21 A, Helsinki, Finland.