Investigations in the Properties of Tooth Enamel by Means of X-Rays.

By

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One of the authors (A.T.J.) of the following has for some time been studying the chemistry of calcium phosphates, structure analyses of crystals and the X-ray determinations of particle size.

These studies have led to conjectures as to the constitutions of tooth enamel and, where opportunity offered, experiments have been made. It is our hope that the experiments and conjectures, however fragmentary, may yet contain facts which will prove useful to odontologists.

Introduction.

Of late years X-ray crystallography has widened our scope of knowledge of those calcium phosphates which comprise the inorganic part of bone tissue as well as the pathological calcium phosphate deposits in the human organism. Three facts of special importance have been discovered.

1) Bone phosphate, dental enamel, dentin and practically all pathological calcium phosphate deposits are composed of *apatite*. They show the same powder diagram as do minerals of the apatite group. (DE JONG (5), ANDRESEN and GOLDSCHMIDT (6), TOVBORG-JENSEN and THYGESEN (7).

The apatite group is a well-defined mineral group of which fluor apatite with the formula $Ca_5(PO_4)_3F$ is the most important representative. The composition of most of the natural apatites follows no single formula. However, the composition of the majority of the well crystallized apatites may be explained by consi-

dering them to be fluor apatite in which varying amounts of OH and Cl entered instead of F and quantities of other bi-valent metals are substituted for Ca.

2) Particles of bone apatite, dentin apatite and enamel apatite have colloidal dimensions. BALE, HODGE and WARREN (8) have determined the particle size of enamel apatite to be 270 Å. (An Å or *Ångström unit* $= 10^{-8}$ cm). The particle size of bone apatite was given as one tenth of this. According to THEWLIS (9) the thickness of the particles of tooth enamel is about 1000 Å.

That particles of bone and tooth apatite have a very slight degree of thickness is seen from the fact that the lines in their powder diagrams are broader than the lines in a diagram of well-crystallized mineral apatite. From this broadening of lines it is possible to compute the average particle thickness of tooth enamel. Fig. 1 shows a powder diagram (A.T.J.) of a particulary well crystallized mineral apatite. Note the sharp lines. Fig. 2 shows a diagram of tooth enamel powder. The lines in this diagram are thicker than in the foregoing, but the similarity between the two diagrams is striking and proves the close relationship of the two compounds.

The determination of particle size by X-rays is only applicable to crystalline particles and only in the submicroscopic or "colloidal" interval. This interval ranges roughly from 3000 to 20 Å.

3) It has finally been proved that the tiny apatite particles in dental enamel are not distributed at random but have a certain degree of orientation, i.e. the majority lie with their crystallographic C-axes nearly parallel. A and B axes of the individual crystals are orientated at random. That axis, of which the direction is fairly determined (in this case the C-axis) is called the fibre axis of the system. The expressions fibre structure and fibre axis do not indicate that we assign to tooth enamel the *properties of fibres*. The terms merely indicate the presence of a particle orientation as described and owes its origin to the fact that said orientation was first discovered in textile fibres (cotton etc.) (HERZOG, JANCKE, POLANYI (11).

In dentin and bone on the other hand, the apatite particles have absolutely no preferential orientation.

THEWLIS (10) to whom we entirely owe our knowledge of the orientation of enamel particles, has demonstrated that all particles in canine enamel have but one orientation, the fibre axis being perpendicular to the surface.

Fig. 1. Powder diagram of mineral apatite.

Fig. 2. Powder diagram of tooth enamel.

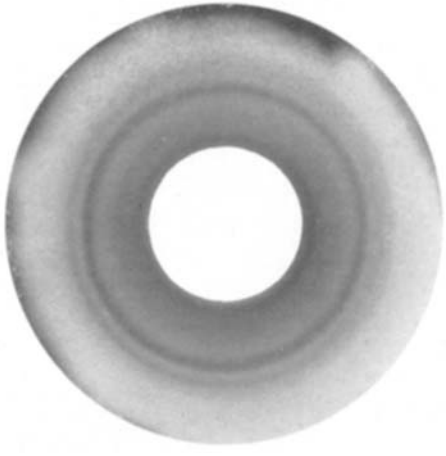

Fig 6. Reflection diagram of enamel from an intact surface.

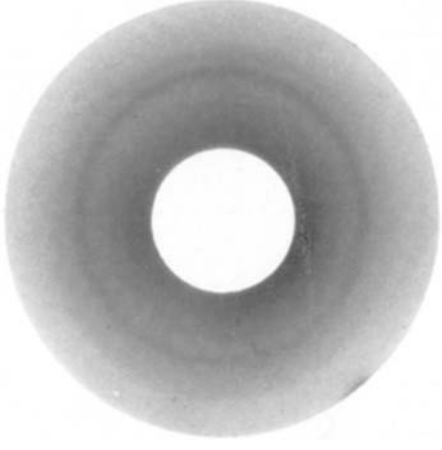

Fig. 7. Reflection diagram of enamel from a ground surface.

Fig. 8. Reflection diagram of synthetic hydroxylapatite.

Human enamel on the other hand contains two distinct kinds of oriented crystals (vide Fig. 3). One kind (i) whose fibre axis is tilted 20° from the surface normal N away from the root, another kind (ii) whose fibre axis is tilted 10° from the surface normal but towards the root. We do not know whether these two kinds of particles are intimately interleaved or are grouped separately in the enamel.

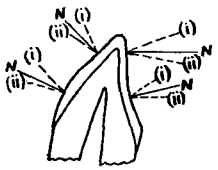

Fig. 3. Orientation of apatite particles in human enamel (THEWLIS).

Is the carbonate content of teeth to be found within the apatite particles or outside?

The diversity of composition of mineral apatites is not exhausted by the formula and rules of substitution mentioned above. Nature is rich in apatites (especially micro-crystalline apatites) which in addition contain lesser amounts of carbonate, alkali metals, and rare earth metals. The form under which these elements are present in mineral apatite is much discussed. The focal point of the discussion is the carbonate content of apatite. This question is also important in understanding certain dental problems since enamel, and especially dentin, deviate in composition from hydroxylapatite $Ca_5(PO_4)_3OH$. The degree of neutralization Ca/P (the ratio between equivalents of calcium and molecules of phosphoric acid in the compound) is below $3^1/_3$ which is the degree of neutralization in hydroxylapatite. Moreover dentin and enamel contain some carbonate. According to ARMSTRONG'S review of the literature (1) enamel contains about 3 per cent of carbonate.

The carbonate content in mineral apatites may be explained in two ways. The first explanation is that of GRUNER and MCCONNELL (12), (13). They postulate that the carbonate enters the apatite lattice but in a way contrary to the accepted rules of crystal chemistry. The second is advocated in particular by THEWLIS (14). He states that extraneous matter does not enter the apatite lattice but remains (1) either amorphous and adsorbed to the surface of the apatite particles, or (2) crystallized in the cracks and crevices in and between the apatite crystals.

THEWLIS believes that carbonate containing apatite *may* contain crystalline calcium carbonate (calcite or aragonite) although neither he nor others have demonstrated the presence of calcium carbonate lines in the powder diagram of carbonate apatite. He believes this because neither NIGGLI and BRANDENBERGER in 1934, nor he himself in 1939 have been able to show smaller admixtures of calcium carbonate to apatite than 10 per cent. We (the authors) have repeated these experiments. We have taken diagrams of mixtures of colloidal apatite and calcium carbonate. The size of the apatite particles was roughly the same as in tooth enamel. The technique used (camera diameter 191 mm, hydrogen filled, filtered cobalt radiation) proved superior to the technique of NIGGLI and THEWLIS. A diagram of 90 per cent apatite and 10 per cent calcite contained 22 calcite lines, 95 per cent + 5 per cent yielded 5 calcite lines and $97^1/_2$ per cent + $2^1/_2$ per cent yielded 3 calcite lines, of which one (the reflection 112 rhombohedral axes) was of medium strength. Using this line it is possible to demonstrate the presence of 1 per cent calcite in apatite.

The second calcium carbonate modification, aragonite, requires a somewhat larger percentage for demonstration. A mixture of 95 per cent apatite and 5 per cent aragonite yields nine aragonite lines. Six of these, however, are "very weak", three "weak-to-medium". Using these 3 lines it becomes possible to find 2 per cent aragonite.

The authors had no opportunity to examine mineral carbonate apatites. However a series of enamel and bone diagrams proved on careful examination to be free of calcite and aragonite lines. The carbonate content in enamel dentin and bone phosphate represent respectively 5—7 per cent $CaCO_3$, 6—8 per cent and about 10 per cent. 1 per cent calcite and 2 per cent aragonite may, as stated, be found by X-rays. Hence the carbonate present in enamel, dentin and bone must exist under another form than crystalline $CaCO_3$.

Two possibilities remain. 1) Calcium carbonate is present in the amorphous state adsorptively bound to the surface of the apatite particles. 2) Calcium carbonate enters the apatite lattice.

We presume the first explanation to be the more probable in the cases of tooth and bone as well as mineral apatite. Many objections may be raised to the second explanation. Some of these are of a general nature and some more directly applied to the experiments of GRUNER and MCCONNELL.

The first explanation of the carbonate content proposed, that $2OH^-$ are replaced by $1CO_3^{--}$ in the lattice need not be discussed. Space consideration precludes this. The recent GRUNER—MCCONNELL theory postulates that a C-atom at random points of the lattice replaces a P-atom, at other points a Ca-atom (however with a changed co-ordinate number). To maintain electro-neutrality twice as many P as Ca are replaced. Against this the following objections may be added to those already brought forward.

From a chemical view point the theory is very unprobable. The C-atom which replaces Ca is situated between 3 phosphate O's. These O-atoms are shared by C and P. This probably means that molecular bonds exist between C and O and between O and P. Hence the crystal no longer contains isolated phosphate and carbonate ions but irregular poly-carbonate-phosphate-ions. Such ions are rather improbable. To be sure crystals with P-O-P bonds (pyro and poly phosphate) and crystals with P-O-X bonds (for instance $ALPO_4X = Al$) are known. Analogous C compounds are not known, nor are polycarbonates known. The theory requires the C-O distances in these C-O-P grouping to be circa 20 per cent larger than in an ordinary carbonate ion: This is highly improbable. The distance Si-O has been shown to be the same in isolated ortosilicate ions as in polysilicate ions which contain Si-O-Si bonds. An analogous condition ought to prevail in carbonates.

From a chemical view point no decisive objections can be made to the claim that dental enamel, etc, contains 5—10 per cent $CaCO_3$ adsorptively bound to the particle surfaces. The particles are so minute that a large percentage of the apatite is located on the surface-layer of the particles. Synthetic colloidal apatite is able to bind rather large quantities of extraneous matter.

Colloidal hydroxyl apatite prepared by precipitation at 100° in alkaline solution has similar particle dimensions to those of enamel and binds water very strongly. After drying at 110°C, it still retains 6—7 per cent water, i. e. 1.8—2.1 mols H_2O per mol $Ca_5(PO_4)_3OH$.

Furthermore there are many indications that also mineral "Carbonate apatites" are colloidal and therefore able to bind much extraneous matter adsorptively. One of the authors (A. T. J.) has shown that certain opaque "single crystals" of mineral apatite are composed of coherence intervals so small that they give powder diagrams with broad lines. However the difference between a

powder diagram of "large" apatite particles (particle size > 3000 Å) and one of medium particles (size f. inst. 3000 Å) first becomes apparent when working with higher dispersion than usual. The difference between "sharp" and "diffuse" lines is striking when working with a camera diameter of 191 mm. as employed by the authors. It is scarcely perceptible in an ordinary 57.3 mm camera as the one employed by GRUNER and MCCONNELL.

GRUNER and MCCONNELL have only indexed their diagrams taken with Fe-Kα radiation up to $\theta = 43°$. They tabulate 7 reflections with higher θ than 43°, but not the indices of these reflections. Up to $\theta = 43°$, even quite distinctly colloidal apatites (tooth enamel, phosphorite and hydroxylapatite precipitated at 100°) give distinct and measurable lines. The strong broadening first sets in at higher values of θ.

The material published seems rather to indicate that the mineral carbonate apatites examined were colloids. These minerals deserve a closer investigation.

Based on the objections of THEWLIS, GLOCK and MURRAY (10) to carbonate substitution, and on those discussed above, we may say that the existence of mineral carbonate apatites is still unproven. Therefore there is little reason for odontologists to believe that the carbonate in teeth is a part of the apatite lattice, — the less so, in that the particle size in dentin and enamel is so small that 3.3—6.2 weight percentage $CaCO_3$ corresponding to 2—4 percentage CO_2 may easily be adsorptively bound to the surface of the particles.

With what degree of accuracy are the lattice constants for dental enamel and hydroxyl apatite equal?

THEWLIS, GLOCK and MURRAY (10) state that synthetic hydroxyl apatite, canine and human enamel have the same lattice constants, viz. a = 9.41 Å, c = 6.87 Å. The probable error, however, is not stated, but will hardly exceed 0.01 Å.

BURRI et al. (15) describe a mineral hydroxyl apatite from Hospenthal, Canton Uri, Switzerland (of correct composition, corresponding to the formula). They give a = 9.42 Å but c = 6.935 Å.

Because of this discrepancy, and because the exact lattice constants for enamel may become important for the reflection technique dealt with in the next section, the lattice constants of

synthetic hydroxyl apatite and a few samples of human enamel were determined.

A circular camera was employed, diameter 191 mm, rectangular slit, reference knives according to PHRAGMÉN-BRADLEY, calibrated with NaCl.

Hydroxyl apatite was precipitated slowly at 100°. pH = ca. 10. A solution of $CaCl_2$ and a solution of sodium phosphate (corresponding to $Na_{10}(PO_4)_3OH$ were added to a large amount of water. The precipitate was washed free of chloride on the centrifuge, dried and ignited. The procedure gives an hydroxyl apatite of great purity.

The lattice constants of the hydroxyl apatite were found to be a = 9.431 Å c = 6.857 Å, in excellent agreement with THEWLIS, but deviating greatly from BURRI. The same value was found for dental enamel, i. e. the same value of glancing angles were found for enamel and hydroxyl-apatite, but as enamel is a colloid, and the lines of large θ cannot be measured, the lattice constants of enamel are determined with less accuracy. We surmise the inaccuracy of the lattice constants of hydroxyl apatites to be 1 : 5000 of dental enamel 1 : 1000.

The result found furnishes further circumstantial evidence for the claim that dental apatite is hydroxylapatite with no content of carbonate ions in the lattice.

The investigation of particle size and orientation in dental enamel may become more exact by using a reflection technique.

THEWLIS, in his important investigations of particle orientation in enamel, used a transmission technique (Fig. 4). The diameter of the primary beam was circa 0.5 mm. A diagram obtained with this technique reveals an average of conditions within a layer of enamel extending from the surface to at least a depth of 0.5 mm. This is a very thick layer in proportion to the dimension of the apatite particles. If in agreement with BALE et al. (8), we take the particles to be 270 Å thick, we easily see that in 0.5 mm enamel there are 18,000 layers of crystals. We must therefore consider the possibility that particle size and orientation change with distance from the surface. The researches of THEWLIS and of BALE are not necessarily the final word on the subject. Cf. the discussion as to whether the outer surface of the enamel is a hypercalcified layer 0.1 mm thick (1).

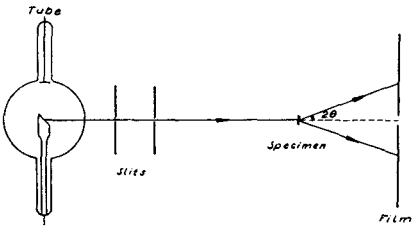

Fig. 4. The principle of the transmission technique.

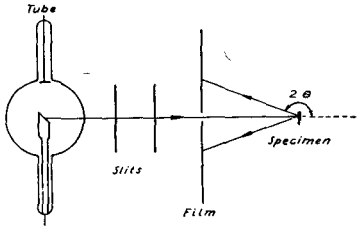

Fig. 5. The principle of the reflection technique.

Much thinner layers may be examined by reflection technique (Fig. 5). X-rays from a target of one of the metals in the series from copper to chromium are strongly absorbed in apatite. For each category of radiation apatite has a characteristic halving thickness. This thickness is the distance an X-ray beam has travelled in apatite when its intensity is reduced 50 per cent (formulae and constants vide (2) page 300 ff.). Some values of halving thickness follow.

Radiation	Wave length	Halving thickness
Cu K_α	1.537395 Å	2.7 10^{-2} mm
Fe K_α	1.932076 Å	1.4 10^{-2} mm
Cr K_α	2.28503 Å	0.8 10^{-2} mm

For the first approximation we can assume that particles lying below halving distance have but slight influence on the characters of the diagram, since their total contribution to the reflected intensity is only 25 per cent.

Those layers examined by reflection technique are then 20—75 times as thin as the layers examined by the transmission technique as applied by THEWLIS. Moreover the method gives more reliable values of particle size than the transmission method. This is because the broadening of lines which there determines the particle size is slight in proportion to the natural breadth of lines (short-wave radiation, small deviation angle, thick specimen), but large in proportion to the natural breadth of lines in the reflection method (long-wave radiation, large deviation angle, small effective specimen thickness). Figs 6, 7 and 8 illustrate this. The figures are reflection diagrams of intact surface enamel (6), slightly ground surface enamel (7) and synthetic hydroxyl apatite (8). We note that there are but few lines on the enamel diagrams, because the crystals have approximately the same orientation. The apatite

diagram contains many lines, the crystals are orientated at random. We note further that the diagrams of intact surface enamel have sharper lines than enamel whose surface has been ground away. The particles in the outermost layer are therefore larger than those lying deeper.

The diagrams are made with a camera originally constructed for another purpose, to take reflection diagrams of metal foils. Therefore the diagrams could not give further information. If such a diagram is to be used for exact determination of particle size, principles of orientation and degree of orientation it must be possible to measure the distance from film to tooth surface exactly. Moreover the orientation of the enamel plate with respect to the film must be registered. Then too, we must be able to tilt the enamel plate at any desired angle from its original position in order to take new diagrams in the new position. One single reflection diagram does not always suffice to give complete information about particle orientation, especially if the specimen, as in human enamel contains particles of different orientation. A camera which fulfills these requirements should not be difficult to construct. With such a camera the desired refinement of THEWLIS' results can be obtained. A closer investigation of the change in properties with distance from surface which the enamel undergoes and a settlement of the discussion of the hypercalcified surface layer could be made by grinding away step by step and investigating each successive layer.

The method should be applicable to investigations of thin hypoplastic enamel from which it is difficult to cut sections. It could also be used in studying enamel from small animals' teeth, because it is much easier to investigate intact teeth than to cut and mount sections.

Particle sizes.

We must emphasize the fact that particle size of dental enamel is not one single figure which may be determined accurately once for all. We have already seen that particle size in enamel diminishes when we leave the surface. The fact that enamel is not a monodisperse system was made plain to us when two different enamel specimens (both 10 mg filings of front surface of two front teeth) yielded diagrams of which one had definitely broader lines than the other. The experiments were not continued for we realized

that an accurate particle size determination according to JONES (16) or according to the method proposed by one of the authors (A. T. J. 17) of the present paper had no particular interest, unless we knew where the sample was drawn, position on the tooth and depth. A particle size determination of the enamel *in situ* is greatly to be preferred to an examination of ground filings. A reflection diagram will give a figure which measures the average particle size of the enamel in a space $^1/_2$ mm in diameter and a few hundredth of a mm thick. Such a space weighs about 0.01—0.02 mg. For comparison we know that if a powder is ground and gummed to a glass rod, manual dexterity is necessary in working with such relatively large amounts as 3—4 mg.

Summary.

There is an introductory summary of the present knowledge of the crystal structure of dental phosphates. Follows a thorough discussion of how the carbonate in dental phosphate is present. It is proved experimentally that the carbonate cannot be present as crystalline $CaCO_3$. Objections are raised to a theory that carbonate ions enter the apatite lattice. The conclusion is drawn that the carbonate in dentin and enamel presumably appears as amorphous $CaCO_3$, adsorptively bound to the surface of the colloidal apatite particles. The lattice constants of hydroxyl apatite $Ca_5(PO_4)_3OH$ were found to be a = 9.431 Å, c = 6.857 Å $\pm$ 1 : 5000. It was seen that tooth enamel has the same lattice constants as hydroxyl apatite, however $\pm$ 1 : 1000. This furnishes circumstantial evidence that dental apatite is hydroxylapatite without carbonate ions in the lattice. Finally it is pointed out that a reflection technique may be used to determine particle size and orientation in areas of enamel which are very much smaller than those in which the transmission or grinding technique is used.

Zusammenfassung.

Einleitungsweise wird ein Übersicht über unser gegenwärtiges Wissen von dem Feinstruktur der Zahnphosphate gegeben. Darauf wird eingehend erörtert, wie der Gehalt von Karbonat in Zahnphosphate vorkommt. Es wird experimentell bewiesen, dass das Karbonat als kristallinisches Kalziumkarbonat nicht vorhanden

sein kann. Einwände gegen die Anschauung, dass das Karbonation im Apatitgitter vorkommen solle, werden gemacht. Es wird konkludiert, dass das Karbonat in Dentin und Emaille wahrscheinlich als amorphes Kalziumkarbonat, zur Überfläche der kolloiden Apatitpartikeln adsorptiv gebunden, vorkommt. Als Gitterkonstanten des Hydroxylapatits $Ca_5(PO_4)_3OH$ stellen sich heraus: a = 9.431 Å c = 6.857 Å $\pm$ 1 : 5000. Es wird gesehen, dass die Zahnemaille dieselbe Gitterkonstanten wie der Hydroxylapatit hat, jedoch ist die Unsicherheit 1 : 1000. Dieses ist ein weiteres Indiz dafür, dass die Apatitpartikeln in der Zahnemaille Hydroxylapatit ohne Karbonationen im Gitter sind. Endlich wird darauf hingewiesen, dass durch die Rückstrahlungstechnik die Partikelgrösse und -orientierung in Zahnemaillegebieten bestimmt werden kann, die um vielhundertmal kleiner sind, als wenn die Durchstrahlungstechnik verwendet wird.

Résumé.

Le traité s'ouvre par un exposé des données que nous possédons actuellement sur la structure cristalline des phosphates dentaux. Suit une discussion serrée sur la présence du carbonate dans les phosphates dentaux. Il est démontré expérimentalement que le carbonate ne peut pas être présent sous forme de carbonate de calcium cristallin. Il est soulevé des objections contre la théorie que l'ion de carbonate ferait partie du réseau d'apatite. L'auteur conclut que le carbonate entre probablement dans la composition de la dentine et de l'émail à l'état de carbonate de calcium amorphe lié par un procès d'adsorption à la surface des particules d'apatite colloidales. Les constantes reticulaires de l'apatite oxhydrile $Ca_5(PO_4)_3OH$ sont fixées à a = 9.431 Å, c = 6,857 Å avec un écart de 1 : 5000 environ. Il est démontré que l'email dentaire possède les mêmes constantes réticulaires que l'apatite oxhydrile, bien qu'avec un écart de 1 : 1000. Ceci tend à prouver ultérieurement que les particules d'apatite de l'émail dentaire sont constituées par de l'apatite oxhydrile sans ions de carbonate dans le réseau. Il est signalé en dernier lieu qu'au moyen de la technique de réflexe il est possible de déterminer l'étendue et l'orientation des particules dans des secteurs d'email dentaire plusieurs centaines de fois plus petits que par l'application de la technique de transmission.

References.

It has not been the intention of the authors in the introductio n to summarize the complete literature on the subject. A summar y of the works published in recent years (1934—42) is found under (1). The principles of X-ray crystallography, powder diagrams and fibre diagrams are treated exhaustively in (2) and (3), from a popular point of view, and in Danish (4).

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