Fraction precipitating between 30 and 50% $(NH_4)_2SO_4$ saturation

	early gastrula stage 11		neurulation stage 14		tail bud stage stage 18
B × B	B	→	B	→	BV
V × V	V	→	V	→	BV
V × B	B	→	B	→	BV
B × V	B	→	B,V	→	BV

Fraction precipitating between 50 and 70% $(NH_4)_2SO_4$ saturation

	late blastula stage 9		early gastrula and neurulation stages 11 and 14		tail bud stage stage 18
B × B	B	→	B	→	BV
V × V	V	→	V	→	V
V × B	BV,V	→	BV,V	→	B,V
B × V	BV,V	→	BV	→	BV

Fraction precipitating between 70 and 90% $(NH_4)_2SO_4$ saturation present only since the tail bud stage

B × B	B
V × V	V
V × B	B,V
B × V	B,V

Results and interpretation:

B × B: *B. bufo;* V × V: *B. viridis;* V × B: *B. viridis* ♀ × *B. bufo* ♂; B × V: *B. bufo* ♀ × *B. viridis* ♂; the stages are those taken from the book by RUGH[3].

B = precipitation by the anti-bufo serum;

V = precipitation by the anti-viridis serum;

B,V = precipitation by anti-bufo serum, after precipitation by anti-viridis serum and viceversa (two different molecules);

BV = precipitation by anti-bufo and anti-viridis sera, but not two precipitations of the same sample (two reactive groups in the same molecule);

BV,V = precipitation by anti-viridis serum after precipitation by anti-bufo serum but not *viceversa* (possible interpretation: two kinds of molecules are present both with reactive groups for anti-viridis, but only one with reactive groups for anti-bufo).

and successively with the other one: in order to recognize whether the reactive groups are located in two different molecules. Two results are possible: 1. the extract, precipitated by the first antiserum, does not precipitate with the second one: the two reactive groups seem to be located in the same molecule; 2. the extract is precipitated by the first antiserum, and then also by the second one: two kinds of molecules seem to be present one for the first reactive group(s), the other for the second one(s).

Molecular ontogenesis. Antigens (or groups of antigens) are present in *B. bufo* embryo during gastrulation and neurulation: they are precipitated from a solution in Weber and Edsall fluid at a concentration of ammonium sulphate between 30 and 50% saturation and 50 and 70% saturation: these fractions are precipitated by anti-bufo serum, but they are not precipitated by anti-viridis serum. The precipitation by anti-viridis serum starts at the tail bud stage. It is impossible to precipitate these fractions by anti-viridis serum after precipitation by anti-bufo serum or by anti-bufo serum, when precipitated by anti-viridis serum. If we call B the molecules with a reactive group for anti-bufo serum, V the molecules with a reactive group for anti-viridis serum and BV the molecules with both reactive groups, we can assume that during gastrulation and neurulation B is present, while at the tail bud stage, B disappears and BV is present.

In the fraction, precipitating at a concentration of ammonium sulphate between 30 and 50% saturation, of the viable cross *B. bufo* ♀ × *B. viridis* ♂, a different ontogenesis of the antigens was discovered. At the early gastrula stage, only the antigens B were present. At the neurula stage, both antigens B and V were present. But at the tail bud stage, only BV antigens were present.

Molecular genetics. Reciprocal crosses were used to study the gene actions at a molecular level. In the fraction, precipitating at a concentration of ammonium sulphate between 50 and 70% saturation, of the viable cross *B. bufo* ♀ × *B. viridis* ♂ only antigens BV could be detected after the early gastrula stage, but these antigens were absent at the tail bud stage of the lethal cross *B. viridis* ♀ × *B. bufo* ♂. It is possible to detect in this cross at the tail bud stage antigens B and V represented by different molecules. We can, therefore, show evidence of a second characteristic of the lethal cross. The lethal cross is unable to combine the two reactive groups in the same molecule, whereas this possibility exists in the reciprocal cross. The other characteristic of the lethal cross is that some antigen(s) starts only with the paternal reactive group (antigen precipitating at a concentration of ammonium sulphate between 30 and 50% saturation, see the Table and [1,2]). The incapacity to combine the two reactive groups in the same molecule can be related to the low synthetic activities well known in the lethal crosses of the amphibians.

Riassunto. È stata studiata la comparsa di antigeni nel corso dello sviluppo di due specie di rospi: *Bufo bufo* e *B. viridis* e dei loro incroci reciproci. Alcuni antigeni al loro primo apparire precipitano solo con l'antisiero specie-specifico e, solo dopo lo stadio di bottone caudale, sono precipitati anche dall'antisiero dell'altra specie; questo viene interpretato quale una ontogenesi molecolare. Negli incroci, le molecole con gruppi reattivi per i due antisieri sono più frequenti nell'incrocio vitale (*B. bufo* ♀ × *B. viridis* ♂), mentre l'incrocio letale (*B. viridis* ♀ × *B. bufo* ♂) mostra alcuni antigeni separati che precipitano ognuno con un antisiero.

S. RANZI, P. CITTERIO, and C. SAMUELLI[4]

Istituto di Zoologia, Università statale, Milano (Italy), October 7, 1960.

[3] R. RUGH, *Experimental Embryology* (Burgess, Minneapolis, Minn. 1952).

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Electron Diffraction and Electron Microscopy Studies on the Crystalline Component of Enamel of the *Odontaspis* (Selachii)

The nature and structure of fish tooth enamel present many problems and some points as yet not well understood. Some authors[1,2] consider fish enamel as a true enamel, but others believe it is a modified form of dentin which they call 'vitrodentin'[3] or 'fibrodentin'[4]. Due, also, to the great interest from the histogenetic point of view[5],

[1] C. TOMES, *Traité d'anatomie dentaire – Humaine et comparée* (Octave Doin, Paris 1880), p. 88.

[2] T. W. WIDDOWSON, *Special or Dental Anatomy and Physiology and Dental Histology*, Vol. II, 7th Ed. (Staples Press Ltd., London 1946), p. 67.

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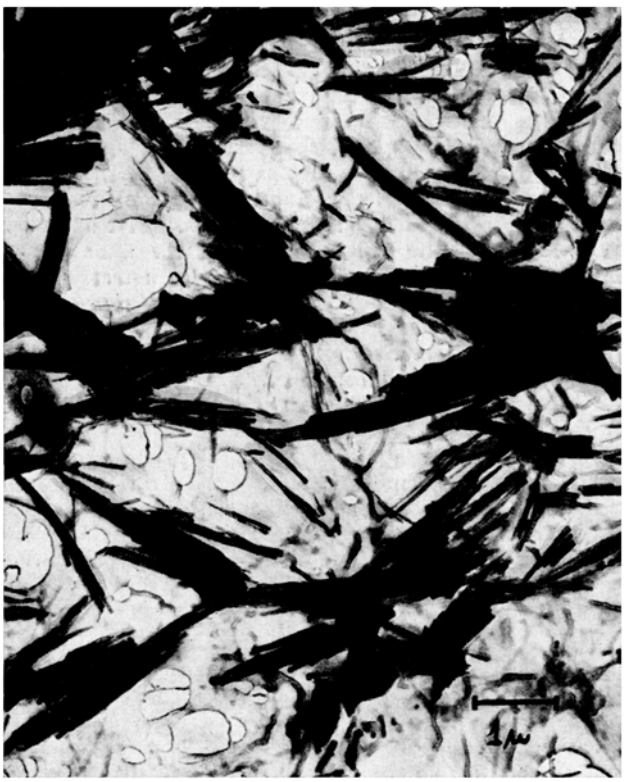

Fig. 1. Positive pseudo-replica of shark enamel showing the rod-shaped crystalline component (Longitudinal section).

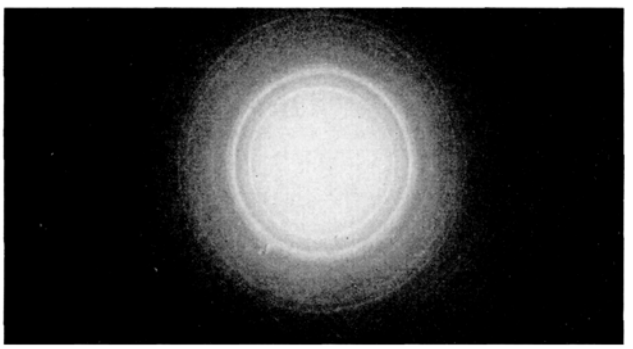

Fig. 2. Typical electron diffraction pattern of the pseudo-replica of shark enamel, shown in Figure 1.

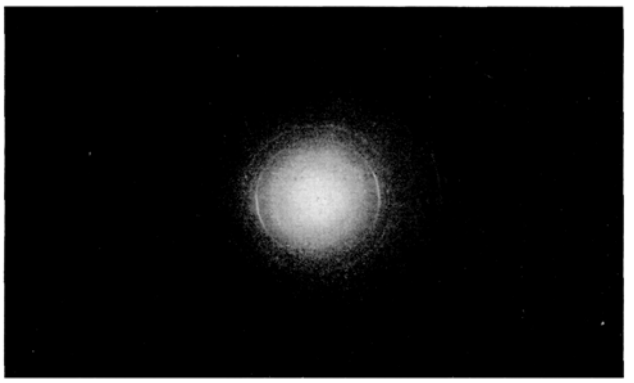

Fig. 3. Electron diffraction pattern of a pseudo-replica of the shark enamel showing preferential orientation.

the authors have begun[6] a systematic study on the ultrastructure of shark's teeth from a common shark from the Brazilian coasts, *Odontaspis*. The ultrastructure of the substance covering the shark dentin was found morphologically to be very similar to human enamel[6,7]. The present note describes the results of studies by electron diffraction and electron microscopy on the crystalline component of enamel of the shark *Odontaspis*.

Throughout this work, positive replicas of transverse and longitudinal sections, as described by AUSTIN and SCHWARTZ[8], were used. In this kind of replication, original tooth material, in the form of a very thin layer of loosened crystals from the enamel, is incorporated on a plastic film constituting what is called a pseudo-replica, which can be successfully studied by electron diffraction. Pseudo-replicas of convenient thickness were prepared for electron microscopy and for electron diffraction and the thinner ones were submitted to electron diffraction. An electron microscope RCA EMU type, equipped with both limited area and standard electron diffraction attachments, was employed. Figure 1 is an electron micrograph of the crystalline component of the shark enamel; Figure 2 is a typical powder electron diffraction pattern of the same component; Figure 3 is an electron diffraction pattern of another pseudo-replica of the crystalline component showing preferential orientation.

The crystalline component of the shark's teeth enamel is morphologically very similar to the crystal-like component[9] of the human enamel. It is composed of bundles of thin rods arranged parallel to their length, ranging from 200 to 900 Å in diameter and of variable length, as shown in Figure 1, which is a pseudo-replica of a longitudinal section of the shark's teeth.

These pseudo-replicas, containing the bundles of thin rods, when examined by electron diffraction either by limited area or by the standard attachment, showed a typical interference pattern (Figure 2) of a crystalline substance which indicates that the rods are crystals.

Line number	$d_{Å}$	$I_{Å}$	d_{R}	I_{R}
1	3.42	5	3.51*	8
2	3.13	2	3.14	1
3	2.79	10	2.78*	10
4	2.695	2	2.36	2
5	2.609	2	2.14	2
6	2.237	5	1.87	5
7	1.933	5	1.73*	5

$d_{Å}$ = spacings in Å from natural apatite.
d_{R} = spacings in Å from pseudo replicas of shark enamel.
$I_{Å}$ and I_{R} = estimated intensities.
* = lines showing preferential orientation.

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[11] D. B. SCOTT, J. R. micr. Soc. *75*, 217 (1956).

Measurements of the spacings and relative intensities from the electron diffraction patterns are shown in the Table. In this Table are also shown the X-ray diffraction data of natural apatite[10]. The comparison of both data have satisfactorily shown agreement in the spacing values and in the relative intensities; therefore it follows that the crystalline component of the shark enamel has the same structure of the mineral apatite as is present in human enamel. As occurs in human enamel[11], electron diffraction patterns clearly showing preferential orientation of the crystalline component were also obtained (Figure 3). The enamel of the shark *Odontaspis*, having its ultrastructure very similar to the human's enamel and having been now established the identification of its crystalline component as natural apatite, can be concluded to be a true enamel.

The ease with which the crystalline apatite of the shark *Odontaspis* is detached from the enamel in tooth sections makes it a very convenient material for the study of its ultrastructure, as well as a suitable source of tooth apatite for crystallographic studies. Studies to establish the detailed orientation of these rods in the shark tooth enamel is under course and will be published elsewhere.

Résumé. L'auteur constate que dans la dent du Requin du genre *Odontaspis* l'ultrastucture de la substance qui recouvre la dentine est morphologiquement très semblable à celle de l'émail de la dent humaine. Par diffraction électronique le composant cristallin de l'émail du Requin a été déterminé comme étant de l'apatite. Ces résultats montrent que le revêtement externe de la dent d'*Odontaspis* peut être considéré comme un véritable émail.

HELENA DE SOUZA SANTOS and
W. DA SILVA SASSO

Electron Microscopy Section, Polytechnical School, University of São Paulo; Department of Histology, Faculty of Farmacy and Odontology, University of São Paulo (Brasil), August 15, 1960.

Bildung von 17α-Hydroxy-Δ^5-pregnenolon und 3β-Hydroxy-17-keto-Δ^5-androsten (DHA) in Nebennieren- und Testes-Gewebe[1]

Kürzlich berichteten wir über das Vorkommen einerseits von 17α-Hydroxy-Δ^5-pregnenolon (3β,17α-Dihydroxy-20-keto-Δ^5-pregnen) in Schweinenebennieren und anderseits von DHA (3β-Hydroxy-17-keto-Δ^5-androsten) in Stierhoden[2]). Diese Befunde liessen zum ersten darauf schliessen, dass der über die Δ^5-Steroide führende Biosyntheseweg der Androgene nicht, wie angenommen[3], ausschliesslich für Nebennieren gilt, sondern auch für Hodengewebe; letzterer Schluss ist nur stichhaltig unter der Voraussetzung, dass das aufgefundene DHA nicht beigemischtem neoplastischem Gewebe entstammte[4]. Zum andern war bei der bekannten Parallelität zwischen DHA-Ausscheidung und Nebennieren-Funktion das offensichtliche Fehlen von DHA in normalem Nebennieren-Gewebe[5] und Nebennieren-Venenblut[6] noch kein Beweis dafür, dass es in der Nebenniere überhaupt nicht gebildet werden kann und ausschliesslich als peripheres Abbauprodukt von adrenalem 17α-Hydroxy-Δ^5-pregnenolon betrachtet werden muss[7].

Wir prüften deshalb, ob normales Nebennieren- und Hoden-Gewebe des Rindes *in vitro* fähig ist, Δ^5-Pregnenolon in 17α-Hydroxy-Δ^5-pregnenolon überzuführen und letzteres zu DHA abzubauen.

Je 100–400 g schlachtfrisches Gewebe wurde, wie früher angegeben[8], bei pH 7,4 homogenisiert und gegebenenfalls in der Kälte fraktioniert zentrifugiert (5000 × *g*), so dass man 2 Fraktionen erhielt: R das Zentrifugat und L die überstehende Lösung. Sowohl Totalhomogenat wie Fraktionen R und L wurden unter Zusatz von 100 mg ATP, 100 mg DPN, 50 mg DPNH, 25 mg TPN und 5 mg TPNH pro 200 g Gewebe zwischen pH 7 und 8,5 während 3 h bei 35°C unter Durchleiten von 1–1,5 l Sauerstoff/min inkubiert. Die Steroid-Vorstufen wurden in 5–10 ml Äthanol gelöst oder suspendiert zugegeben. Die nach Eingiessen der inkubierten Homogenate in insgesamt 16 l Aceton erhaltene Aufschlämmung filtrierte man nach 12 h bei 0°C, wusch den Rückstand mit Aceton, engte die vereinigten Filtrate auf 1,5 l ein und extrahierte sie mit 4 × 800 ml Chloroform. Die meist 2–5 g wiegenden Chloroform-Rückstände wurden an 40–100 g Silicagel Davison adsorbiert und je nach Art des eingesetzten Substrates und des zu bestimmenden Umsetzungsproduktes mit 0,8–2 l Benzol-$CHCl_3$ (1:1) und 0,4–1 l $CHCl_3$-Aceton(1:1) (für 17α-Hydroxy-Δ^5-pregnenolon) bzw. mit 2 l Benzol und 1 l $CHCl_3$-Aceton (1:1) (für DHA) eluiert. Die $CHCl_3$-Aceton(1:1)-Eluat-Rückstände (0,5–2 g) setzte man mit je 1 g Girard-Reagens T und 2 g Amberlite IRC 50 (H^+) in 100 ml Äthanol 20 h bei 20°C um, isolierte auf übliche Weise die bei pH 1 leicht und schwer spaltbaren ketonischen Anteile und chromatographierte sie präparativ auf je 2–20 Blatt Papier mit dem System F/Cy-Be(1:2)[9]. Die den gesuchten Δ^5-Steroiden entsprechenden Zonen wurden eluiert und analytisch im System Bush-B_3, -A und Propylenglykol/Toluol rechromatographiert; die Auswertung erfolgte halbquantitativ durch visuellen Vergleich mit Standardsubstanzen, wobei Farbreaktionen mit $SbCl_3$ (für Δ^5-Steroide) oder alkalischem *m*-Dinitrobenzol (für 17-Ketosteroide) benutzt wurden.

Bei Verwendung von Tritium-markierten Verbindungen verzichteten wir auf die Girardierung, führten aber die Silicagelchromatographie differenzierter aus. Ferner wurde für die Isolierung von markiertem 17α-Hydroxy-Δ^5-pregnenolon, aus den Ansätzen mit Δ^5-Pregnenolon-7α-^{3}H, nach der ersten präparativen Chromatographie eine zweite mit Propylenglykol/Toluol eingeschaltet. Das

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