Use of Articles in the Pachyonychia Congenita Bibliography

The articles in the PC Bibliography may be restricted by copyright laws. These have been made available to you by PC Project for the exclusive use in teaching, scholarship or research regarding Pachyonychia Congenita.

To the best of our understanding, in supplying this material to you we have followed the guidelines of Sec 107 regarding fair use of copyright materials. That section reads as follows:

Sec. 107. - Limitations on exclusive rights: Fair use
Notwithstanding the provisions of sections 106 and 106A, the fair use of a copyrighted work, including such use by reproduction in copies or phonorecords or by any other means specified by that section, for purposes such as criticism, comment, news reporting, teaching (including multiple copies for classroom use), scholarship, or research, is not an infringement of copyright. In determining whether the use made of a work in any particular case is a fair use the factors to be considered shall include - (1) the purpose and character of the use, including whether such use is of a commercial nature or is for nonprofit educational purposes; (2) the nature of the copyrighted work; (3) the amount and substantiality of the portion used in relation to the copyrighted work as a whole; and (4) the effect of the use upon the potential market for or value of the copyrighted work. The fact that a work is unpublished shall not itself bar a finding of fair use if such finding is made upon consideration of all the above factors.

We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
NOTICE: THIS MATERIAL MAY BE PROTECTED BY COPYRIGHT LAW (TITLE 17, U.S. CODE)


Department of Dermatology, University of Brussels, Belgium (Hôpital St.-Pierre, 322 rue Haute, 1,000-Bruxelles)

PACHYONYCHIA

G. ACHTEN AND J. WANET-ROUARD

SUMMARY.—One hundred and two cases of pachyonychia with onycholysis were studied both histologically and histochemically on transverse sections of the extremities of the nail.

Globular structures were found in the ventral nail formed by the hyperkeratosis of the hyponychium which was considerably thickened. They seem to arise in the intercellular spaces. These structures coalesce and enlarge. They react like neutral and acid mucopolysaccharides. These clumps, previously described by Zaias in psoriasis of the nail, are also found in several cutaneous hyperkeratotic diseases. Their dermal origin has not been confirmed.

PACHYONYCHIA is characterized by thickening of the nail. It may assume two different clinical forms: the thickening may involve the nail plate itself or the keratin of the hyponychium and eventually that of the nail bed (Fig. 1). The former is due to acanthosis. In the picture of only slight acanthosis, onycholysis often appears and the vacuolar appearance of the nail plate is a consequence of our study.

Materials and methods

Our study is based on the description of these cases which were not reported: pachyonychia in 63 cases, acanthosis, onycholysis in 59 cases. The study of Zaias (1964) in a dermatology clinic of Montreal was most important in defining this condition.

Methods

Transverse sections of nails of pachyonychia were studied at the University Hospital of Brussels by Dr. G. Achten (1963). They were carried out by Dr. J. Wanet-Rouard, A. Haematoxylin-cosin. Van Geel, Masson. In addition, digestion of the nail plate was carried out in the following solutions:

1. The periwinkle:
   i. Salicylic acid 1% in ethanol
   ii. Acetylation product of HCl and 
   iii. Sulphuric acid 1%
2. The Barlow solution:
   a. The u.v.
   b. Catalyses the halogenation of the hydroxylic groups
   c. Periodic acid with silver nitrate
   d. Periodic acid with hydrosulfuric acid
3. The Alcian blue:
   a. Shows the presence of ti
   b. Produced by the action of enzymes

* We thank...
former is due mostly to trophic disorders and terminates ultimately in onychogyphosis. In the latter, the nail becomes loosened from its bed producing a typical picture of onycholysis (Fig. 2). At this level, the keratin formed may be hard or soft. When it is soft, it will disintegrate, and give rise to a very characteristic lacunar appearance (Fig. 3). This second form of pachyonychia will be the object of our study.

MATERIALS AND METHODS

Materials

Our study included 102 cases of pachyonychia with secondary onycholysis. One of these cases was a congenital anomaly involving all fingers and toes. Three other cases were reported in the same family*. The remaining 101 cases were acquired. Psoriasis, eczema, onychomycosis (candidal and dermatophytic infection of the nails) accounted for 59 cases. There were 43 cases in which no specific cause could be found. Alkiewicz (1964) in a detailed study of this type of pachyonychia used the term "colloidal degeneration" while at the same time admitting the limitations of this term.

Methods

Transverse sections of the tip of the nail were taken by the technique described by Achten (1963).

Haematoxylin-eosin staining was employed for the general histological studies. In addition, different histological techniques were used:

1. The periodic acid–Schiff technique (after McManus and Hotchkiss) was used as described by Lison (1960) to demonstrate the presence of glycoproteins containing 1 : 2 glycol groups. Those substances which were positive for PAS were further studied by the following additional histochemical reactions:
   (i) Salivary digestion to exclude the presence of glycogen.
   (ii) Acetylation using the technique of McManus and Cason (1950), which will hydrolyse OH and NH₂ groups and consequently will block their oxidation by periodic acid. The acetylation process is then reversed in a dilute alkaline medium (0.1N KOH) restoring the stain with PAS (Lison, 1960).
   (iii) Sulphation-induced metachromasia after Bignardi (1939, 1940), Kramer and Windrum (1954): this reaction will cause neutral mucopolysaccharides, mucous and glycoproteins to become basophilic and metachromatic (Pearse, 1968).

2. The Bauer method (chromic acid–Schiff), after Lison (1960).


4. Periodic acid–phenylhydrazine–formazan reaction after Stoward (1963, 1967): the condensation of phenylhydrazine and tissue aldehyde causes the formation of a primary aldehyde arylhydrazine. The diazonium salt replaces the acidic α amino hydrogen to give a tetrazene derivative which rearranges immediately into the formazan. The reaction is positive with some aldehydes but negative for ketones (Pearse, 1968).

5. Periodic acid–pseudo Schiff reaction, after Stoward (1963, 1967). The aldehyde produced by oxidation combines by a different mechanism from that of the true Schiff reaction. The pseudo Schiff reagents are fluorescent substances; they demonstrate the presence of tissue polyaldehydes (Pearse, 1968).

6. The Alcian Blue method: A 1% solution of Alcian Blue was used for 20 min. diluted in sulphuric acid 2N (pH 0.5); hydrochloric acid 0.1N (pH 2.5); a buffer of 0.1M citric acid with 0.2M Na₂HPO₄ (pH 4.5).

* We thank Professor Colomb of Lyon who kindly referred this observation to us.
Alcian Blue stains the SO₄ groups at pH < 1, the COOH groups at 2·5 < pH < 3·5 and the proteins at pH > 3·5 (Gerard, 1968).

7. Dialysed iron method, after Muller (1955). Acid mucopolysaccharides and mucoproteins are stained blue. This reaction depends on the affinity of free acidic groups in the tissues for colloidal Fe+++ at pH 2; a positive reaction is also observed with other chemical substances and is not therefore specific for acid mucopolysaccharides (Lison, 1960).

8. Metachromasia was studied in diluted solutions of toluidine blue: 0·1% toluidine blue solution in a buffer of 0·1M citric acid with 0·2M Na₂HPO₄ (pH 3 and pH 5) and in a solution of 0·1N HCl (pH 1). Metachromasia results from polymerization of the dye, induced by polymerization of the substrate containing acid groups.

At pH < 1·5 it shows SO₄ and PO₄ groups. At pH 1·5 < pH < 4 it shows either carboxyl groups or SO₄ groups. However, in this case the SO₄ groups are partially masked by 1 or more basic proteins. At pH > 4 it demonstrates the presence of weak carboxyl groups. Three additional histochemical reactions were used for the study of acid mucopolysaccharides (Gerard, 1968).

(i) Methylation, after Fisher and Lillie (1954) using a methanol/HCl mixture at 36°C. This reaction esterifies carboxyl groups and hydrolysates sulphated esters (Gerard, 1968).

(ii) Testicular hyaluronidase (Sigma). A 0·5% solution diluted in a buffer of Na₂HPO₄/KH₂PO₄ was used at pH 5·5 for periods as long as 72 hr. This may depolymerize hyaluronic acid as well as A and C chondroitin sulphates (Gerard, 1968).

(iii) Crystallized trypsin (Sigma). A 0·1% solution diluted in a buffer of Na₂HPO₄/KH₂PO₄ was used at pH 8·5 for a time varying from 5 to 15 min at laboratory temperature (Gerard, 1968).

9. Fluorescence method with acridine orange. Acridines precipitate acid mucopolysaccharides giving a bright fluorescence. A combination of acridine orange with different concentrations of sodium chloride distinguished different acid mucopolysaccharides, i.e. hyaluronic acid, chondroitin sulphate and heparin (Saunders, 1969) (from Pearse, 1968).

10. Lipid est.

11. Anisotropy

12. SH grow.

Bennett's Meth.

HISTOCHEM.

The transverse section of the effect of the thickening of the tissue, as formed by hyaluronic acid, is stained with a polyampholyte amorphous yellowish structure we have called, the keratinoid. This structure is stained on the one hand with the counterstaining procedures used (10). These structures are surrounded by non-stained spaces. At the junction of these spaces, the keratinoid structure appears as a solid mass. It contains proteoglycans and is in contact with the surrounding cells, which often appear piled up or "agglomérées" de keratinoides. The aperiphysary areas are stained with the counterstain. These areas will contain more pigment than the keratinoid cells. The keratinoid cells are surrounded by non-stained spaces.

Fig. 4.—Formation of the PAS positive clumps. The arrows show their formation in the intercellular spaces with progressive increase of their size.
10. Lipid extraction method. We have used either a mixture of methanol and chloroform in equal parts for 18 hr., or pyridine for 24 hr.

11. Anisotropy was studied by polariscope examination.

12. SH groups were studied following Bennett’s Method (1961) N-ethylmaleimide at pH 7.4 was used as a specific blocking agent for SH groups (Pearse, 1968).

HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS

The transverse sections demonstrated in the affected nails an important thickening of the ventral nail plate formed by hyperkeratosis of the hyponychium with the formation of eosinophilic amorphous clumps. These structures were round or oval and appeared small; however, some were larger, due to the confluence of several smaller groups. Using the McManus stain, the first stage showed small hyperchromatic globules in the periphery of the cell; they appeared to be located in the intercellular spaces (Fig. 4). These structures enlarge and coalesce, and are capable of attaining quite a large size (Fig. 5). These homogeneous clumps are often piled one on top of another (Fig. 6) giving the appearance of “colonnes grillagées” described by Unna (1879, 1881). Less often they are scattered throughout the keratin of the ventral nail. These structures are surrounded by normal keratin and, in many cases, are separated from each other by empty spaces. At a late stage these empty spaces resemble a honeycomb (Fig. 7).

Steigleder (1950) showed histochemically that the clumps contain protein complexes similar or even identical to glycoproteins. We have tried to obtain more information regarding their nature.

(1) In contrast to the neighbouring keratinized cells these structures are not doubly refractile under polaroscopic
examination and therefore do not contain normal keratin fibres.

(2) The periodic acid–Schiff reaction is always strongly positive (Fig. 5, 6). The Bauer reaction and the u.v. tungsstate method are less positive. The periodic acid–phenylhydrazine and periodic acid–pseudo Schiff reactions are also strongly positive. Several substances give a positive McManus reaction. Complementary investigations made it possible to differentiate the substances and gave the following results:

Amylase digestion has never abolished the positivity of the clumps; therefore the presence of glycogen is excluded.

Acetylation at 60°C for 6 hr. prevents the uptake of Schiff’s reagent; therefore we can conclude that they probably consist of neutral mucopolysaccharides.

Hydrolysis in an alkaline medium after acetylation will restore stain with Schiff’s reagent.

Sulphation which induces basophilia and metachromasia in neutral mucopolysaccharides is positive.

A lipid extraction was performed in order to exclude the presence of PAS positive lipids; the PAS reaction was never abolished. Thus we concluded that the clumps contain neutral mucopolysaccharides.

(3) Staining with Alcian Blue was performed at several pH levels (1, 2, 3 and 5). The amorphous clumps became light blue but the affinity for the dye was irregular. This staining was observed mostly in the peripheral area of the cell, starting at a pH of 2.5.

(4) Toulidine blue used at pH 1, 3 and 5 did not colour the clumps, or coloured them slightly blue, orthochromatically. However, in a few cases, moderate metachromasia has been observed on examination of the sections in water.

(5) The colloidal iron method (Muller) gives a very positive reaction (Fig. 8).

After methylation, Alcian Blue and colloidal iron were negative.

Testicular fat at several long periods enzymatic digestion.

(6) Trypsin periods show digestion.

(7) Fluorescence acidine orange hyaluronic chondroitin sulphate.

(8) The his for revealing groups gives activity to the contrast to the keratin.

Our obs: Alkiewicz an.

From our observations enough to normal keratin.

Similar as described in hyperkeratosis as cutaneous, the nails; structures extended in the formed amorphous material is confirmed Zeitz. The observed these first in the comparative hyperkeratosis.

Fig. 7.—Hyperkeratosis of the hyponychium giving a honeycomb appearance (arrows) (toluidine blue).

Fig. 8.—Transverse section of nail tip. 1. Nail plate. 2. Marked hyperkeratosis of the hyponychium (ventral nail) with the clumps coloured by colloidal iron method.
colloidal iron reactions become negative.

Testicular hyaluronidase, used at several concentrations for long periods, showed partial enzymatic digestion.

(6) Trypsin used for several periods showed little enzymatic digestion.

(7) Fluorescence-method with acridine orange was positive for hyaluronic acid, negative for chondroitin sulphate and heparin.

(8) The histochemical method for revealing the presence of SH groups gives little or no positivity to the clumps in contrast to the surrounding normal keratin.

| PRELIMINARY REACTION | METHOD           | PACHYONYCHIA "CLUMPS"
|-----------------------|------------------|------------------------
| Amylase               | PAS              | ++
| Acetylation           | Bauer            | ++
| Acetylation-KOH       | U.V. Tungstate   | +
| Lipid extraction      | PA-Phenyldrazo -formazan | ++
| Sulphation            | PA-Pseudo-Schiff | +
|                      | PAS              | +
|                      | PAS              | ±
|                      | Alcian blue      | +
|                      | Colloidal iron   | +
|                      | Toluidine blue   | ++

Fig. 9.—Table of the histochemical reactions for neutral mucopolysaccharides.

CONCLUSIONS

Our observations concerning the "colloidal degeneration" described by Alkieczewicz are summarized in Fig. 9, 10.

From our study we may conclude that neutral and acid mucopolysaccharides are present but the latter are not very abundant and are seldom polymerized enough to give metachromasia; and normal keratin is absent.

Similar cellular changes have been described in several states of epidermal hyperkeratosis with parakeratosis such as cutaneous horns, warts and papillomata (Steiner, 1959). Zaia (1969) observed the same clumps in psoriasis of the nails; he considered that these structures were of dermal origin: i.e. that a serum exudate penetrated between the cornified cells and finally formed amorphous clumps. Zaia considers this material to be either serum or serum glycoproteins. As far as the initial localization of this amorphous material is concerned our investigations confirm Zaia's findings. We have observed these small globules appearing first in the intercellular spaces. Our comparative studies of several epidermal hyperkeratotic disorders have further

| PRELIMINARY REACTION | METHOD         | PACHYONYCHIA "CLUMPS"
|-----------------------|----------------|------------------------
| Methylatin           | Alcian blue    | ++
| Hyaluronidase        | pH 5           | +
| Trypsine             | pH 2.5         | +
|                      | pH 1           | ±
| Toolidine blue       | pH 5           | +
|                      | pH 3           | ± or —
|                      | pH 1           | —
| Colloidal iron       | +
| Colloidal iron       | —
| Colloidal iron       | + or ±
| Acridine orange      | +
| Hyaluronic acid      | —
| Sulph, chondroitine  | —
| Heparin              | —

Fig. 10.—Table of the histochemical reactions for acid mucopolysaccharides.
confirmed this initial intercellular localization. We have never found in these epidermal disorders, any globules in the dermis. Thus we cannot establish the origin of the amorphous clumps.

We are not able to localize the structures when they become more voluminous or to say whether they have penetrated or pushed away the surrounding cells. Observations with the light microscope cannot provide a solution to this problem.

REFERENCES


SUMMARY—

The radiographic alterations of the nail are described as they are observed in the volume value normal. After increases in the nail sensitivity to H-thymidine labelling, a large differences are noted.

For years considerable investigations have been initiated by the team of people of information in the field of the skin changes in psoriatic plaque, band-like psoriasis, and developed hyponychia. In this paper, we report on H-thymidine in the nail lives, and the results.