Abstract. Skin biopsies from pseudoxanthoma elasticum (PXE) patients and from normals were examined by X-ray fluorescence to study the mechanism of calcification in PXE lesions. The axillary lesions of the 5 patients all showed an elevated calcium content. Only 4 had increased phosphorus. Sulphur, potassium and chloride contents did not deviate from those of normal axillary skin. Sacral skin from patients did not differ from controls. Calcium apatite cannot constitute the sole deposit in PXE calcification.

Pseudoxanthoma elasticum (PXE) is a genetically determined connective-tissue disease, involving the skin, the eyes, and the cardiovascular system (17). Calcification in the skin lesions has been demonstrated by various methods (4, 6, 7, 10, 11, 12, 13, 14, 15, 18). Electron-microscopic studies have shown changes in elastic fibres, particularly as deposits of calcium salts inside the fibres (4, 11). The calcification appears as thin needles reminiscent of apatite crystals, or as granules. Calcification has been reported as the first visible abnormality in PXE (4, 5, 10, 11).

Glutamic acid and glutamine have been found to be increased in aortic elastin in relation to age and arteriosclerosis (8). An increased content of glutamic acid in PXE lesions has also been reported (19). An increased incorporation of (14C) glutamine has been found in newly synthesized elastin from PXE skin lesions (2, 3). As increase of calcium or increase of phosphate have both been reported as occurring primary to calcification (9, 21) we decided to study the Ca and P content in PXE skin.

An increased hexosamine value as representative of glycosaminoglycans has been reported in PXE lesions (20). It was considered worthwhile to include S determination in our studies, as sulphur might be related to sulphated glycosaminoglycans, which could act as binding sites for calcium ions.

As potassium is known to be related to the intracellular biosynthesis of macromolecules (16), measurement of the K content was also performed. The Cl values always appearing from the X-ray graphs taken were included.

MATERIAL AND METHODS

Skin samples

The tissues studied were age-, sex- and area-matched skin biopsies of PXE patients and normal controls. PXE lesions were present in the axillary, but not in the sacral areas of patients. 5 axillary and 6 sacral biopsies from patients and controls were taken.

To determine wet weight, the biopsies were weighed on an ultramicro Mettler balance immediately after excision. All samples studied were defatted with acetone, acetone/ether (1:1), and ether, and desiccated to constant weight by storing in a stainless-steel vacuum desiccator (Nikotanks, Cat. No. 800). Dry defatted samples were weighed ("dry weight") and the content of water plus lipids was calculated as a percentage of the wet weight of the skin.

X-ray fluorescence analysis was performed on dry defatted samples. Calcium, potassium, chloride, sulphur, and phosphorus were determined on a Philips vacuum X-ray

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Source of tissue</th>
<th>Dry weight/100 Wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>PXE</td>
<td>Axillary</td>
<td>26.42 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Sacral</td>
<td>29.96 ± 0.9</td>
</tr>
<tr>
<td>Controls</td>
<td>Axillary</td>
<td>28.56 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Sacral</td>
<td>28.3 ± 1.0</td>
</tr>
</tbody>
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Table I. Elementary analysis on samples of dry defatted skin biopsies from PXE patients and controls

Contents are expressed in arbitrary units of the element per mg sample

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Source of skin</th>
<th>Ca (μg/mg)</th>
<th>K (μg/mg)</th>
<th>Cl (μg/mg)</th>
<th>S (μg/mg)</th>
<th>P (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PXE</td>
<td>5</td>
<td>Axillary</td>
<td>91.0 ± 2.0</td>
<td>5.2 ± 0.6</td>
<td>1.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Sacral</td>
<td>3.03 ± 0.56</td>
<td>3.93 ± 1.65</td>
<td>1.26 ± 0.49</td>
<td>0.57 ± 0.27</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>Axillary</td>
<td>2.7 ± 0.2</td>
<td>5.2 ± 1.5</td>
<td>1.3 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Sacral</td>
<td>2.3 ± 0.8</td>
<td>3.5 ± 1.6</td>
<td>1.3 ± 0.4</td>
<td>0.49 ± 0.12</td>
<td>0</td>
</tr>
</tbody>
</table>

* One sample: 0. (The result is the average of the other 4).
* The difference 91.0 - 2.7 is statistically significant, *P* < 0.001.

spectrometer (type PW 1540). X-ray determination of an element does not yield information about and is independent of the actual state of the element, for example, what is reported as "calcium" would probably be calcium (II) and "chlorine" would be chloride in the sample. The amount of each element in the sample was expressed in arbitrary units per mg dry defatted sample. Averages ± standard error of the mean are reported. Statistical significance was calculated according to the *t*-test. Ratio of Ca to P content of pure apatite was measured for comparison with the skin samples.

RESULTS

**Weight of dry defatted skin**

As shown in Table 1, comparison of dry weight as percentage of wet weight of skin biopsies removed from PXE patients and normal controls revealed no significant differences between the groups.

**Elementary analysis**

Results are shown in Table II. Calcium content in PXE lesions was markedly increased in relation to corresponding areas of the controls and the sacral skin of both groups (*P* < 0.001).

Phosphorus was only detectable in lesions of 4 out of 5 patients and in none of the controls. It was also not detectable in the sacral area of patients. No significant differences were found for potassium, chlorine or sulphur content, either in relation to disease or to area.

**Ratios between calcium and phosphorus content**

Table III shows the individual results of determinations of Ca and P contents in patients and controls. Determination of Ca and P was also performed on a sample of pure apatite. Ratios were established for pure apatite as well as for the dry defatted samples of skin examined. Ratios obtained with PXE axillary samples 2 and 3 could correspond to apatite, while sample 4 had a higher and sample 5 a lower value. Sample 1 had no detectable P.

**DISCUSSION**

As the increase of calcium was more constant than that of phosphorus in PXE lesions, it seems that the calcium-binding capacity of the diseased skin may be of primary significance in the calcification in PXE. The calcification process in arteriosclerosis has been related to the calcium-binding capacity of the dicarboxylic amino acids (14). The finding of Rodnan et al. (19) of increased glutamic acid, together with our own observation
(2, 3) of increased incorporation of \(^{14}\text{C}\)glutamine in the elastin of PXE lesions may also be related to the increased calcium content of the diseased tissues.

Studies by Armstrong (1) on the amino acid composition of human parotid salivary proteins, which selectively adsorbed hydroxyapatite, have shown as the most significant features high proline, glutamic acid and glycine levels. Our results agree with the hypothesis of Urist as to primary factors in calcification (22). He suggested that the binding of calcium to the organic matrix is a necessary and initial step in calcification and that the binding of phosphorus occurs by ion association only after the binding of calcium. Goodman et al. (10) suggested calcium deposition in PXE to be directly related to the genetic defect. According to these authors a derangement of a regulatory mechanism of calcium homeostasis of calcium producing an abnormal calcium deposition on a normal fibre. Our electronmicroscopic studies of PXE have shown that morphologically normal elastic fibres can calcify. Calcification is probably followed by degeneration of the elastic tissue (4, 5). Our biochemical studies on calcified elastic tissue in PXE showing abnormal biosynthesis (2, 3) together with our morphological studies (4, 5) are in agreement with the former possibility suggested by Goodman et al. (10).

However, we do not reject the possibility of a derangement of a regulatory mechanism of calcification. Glimcher et al. (9) stated that some failure of a control mechanism may occur in certain instances of pathological calcification. Our results, showing a significant increase in calcium content in the lesion area (axillary) in comparison with a non-involved area of the PXE patients (sacral) are in agreement with the report of Finnerud et al. (6). These authors studied gravimetrically the calcium content in material obtained from involved areas of 2 PXE patients, from non-involved areas of one of them, and from 3 normal controls.

Our findings of no differences between the S content of the lesions and the control areas suggest that increased hexosamines (20) and glycosaminoglycans (21), if related to the increased calcium-binding capacity of the skin in PXE, are constituents of unsulphated glycosaminoglycans or glycoproteins. This would be in agreement with the finding of Smith et al. (21) of a higher increase of hyaluronic acid than chondroitin sulfate in PXE skin as compared to that of normal controls. Loss of cell potassium is related to depression of macromolecular synthesis or growth of mammalian cells in culture (16). Our finding of no difference in the potassium content of the tissues studied suggests that the differences observed in the macromolecules studied (2, 3) could not be related to alteration of that element.

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N. Blumenkrantz, Ph.D.
Department of Dermatology
Rigshospital
Blegdamsvej 9
DK-2100 Copenhagen
Denmark

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