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Dichroism with Congo Red. A Specific Test for Amyloid?

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Dichroism mit Kongorot. Eine spezifische Probe für Amyloid?

Zusammenfassung. Spongiosa und junge Haverssche Osteonen von Menschen und Ratten färben sich mit Kongorot an. Im polarisierten Licht zeigen die Kongorot-positiven Abschnitte einen charakteristischen Farbwechsel mit grüner Doppelbrechung. Die Möglichkeit einer generalisierten Amyloidose war in den Experimenten ausgeschaltet.

Corpora amylacea im Nervensystem, in Prostata und Lunge wie die intratubulären lamellären Körper in den Nieren bei Myelom färben sich ebenfalls mit Kongorot an und geben im polarisierten Licht eine stark grün aufleuchtende Doppelfärbung. Diese Ergebnisse lassen darauf schließen, daß die Doppelbrechung mit Grün-Aufleuchten, welche bis dahin als Amyloid-spezifisch angesprochen worden ist, nicht als solche anerkannt werden kann. Das Grün-Aufleuchten bei Kongorot-positiven Substanzen beruht auf der Anwesenheit Glykoproteinmolekülen mit großer Affinität zu Kongorot und bestimmter Textur.

Summary. Alveolar and "young" Haversian bone of humans and rats was found to stain with Congo red. Observations in polarized light showed Maltese cross birefringence with green dichroism. The possibility of systemic amyloidosis was rejected.

The laminated intratubular bodies in the kidneys in multiple myeloma, the cerebral and prostatic "corpora amylacea" and pulmonary microliths are also Congo red positive and give a bright green birefringence in polarized light.

Dichroism is merely the product of glycoprotein molecules that are disposed in an ordered pattern and that have a great affinity for Congo red and other dyes.

The green dichroism that some authors have maintained is a specific characteristic of amyloid is shown by the results reported here not to be a completely reliable index of amyloid.

Introduction

The natural birefringence of amyloid under polarized light is increased by previous staining with Congo red and certain other dyes (DIEZEL and PFLEIDERER, 1959). This birefringence is due to the ordered submicroscopical pattern of amyloid fibrils (COHEN and CALKINS, 1959). DIVRY and FLORKIN (1927) were the first to observe the green dichroism of amyloid deposits stained with Congo red when polarized light is used. Numerous authors repeated the observation (ROMHÁNYI, 1943; LADEWIG, 1945; MISSMAHL, 1955; PFEIFFER, 1953) and lately MISSMAHL (1957) and RAVID *et al.* (1967) have claimed this test to be specific for amyloid.

In the course of an extensive study we have found a green dichroic reaction to be given by structures in which the presence of an amyloid deposit could be ruled out. The specificity of dichroism with Congo red for amyloid is reconsidered in the light of our data.

Material and Methods

Various human bones from normal adults and foetuses and bones of normal rats were fixed in 10% formalin for 24 hours and decalcified with nitric acid (3%) or trichloracetic acid (10%) for variable periods of time.

Human brains and prostate glands which contained "corpora amylacea" were fixed similarly.

Biopsy specimens of human testes containing "egg-cell-like bodies" (HORNSTEIN and HEIDINGER, 1965) were obtained from a male pseudohermaphrodite and from a 9-years-old boy with cryptorchidism.

The lungs of two asthmatic patiens were obtained at autopsy. They contained concentrically laminated microliths.

Kidneys containing laminated intratubular bodies were obtained at autopsy in two cases of multiple myeloma.

The material was embedded in paraffin wax after fixation. Sections were cut 6 microns thick. The staining methods used were: haemalum and esoin; Van Gieson; methyl violet for metachromasia; periodic-acid/Schiff-alcian-blue with maltase treatment; a modified Congo red technique (LILLIE, 1965). Leitz polarization apparatus was used as recommended by HELLER *et al.* (1964).

Results

Spicular bone and "young" Haversian systems were Congo red positive. Mature compact bone was consistently negative. In the long bones of rats the spicules in enchondral ossification areas were also positive. Alveolar bone of the human diaphysis was occasionally positive. With P.A.S.-alcian blue the Congo red positive areas (particularly those in newly deposited osteoid substance) were rich in acid mucopolysaccharides that were stained by alcian blue. This was clearly observed in foetal human bones, in which the spicules resulting from direct ossification and those resulting from indirect ossification showed the dichroism. Typical Maltese cross birefringence with two green and two yellow-red arms seen under polarized light in young Haversian system of adult bones (Figs. 1 and 2). This was observed after decalcification with either nitric or trichloracetic acid. Spicular bone showed patches of green birefringence. This reaction was positive even after prolonged treatment with the alkaline-alcoholic solution when the BENNHOLD (1922) method of Congo red staining was used.

All the laminated bodies examined were strongly Congo red positive (Figs. 3 and 5), and in polarized light they frequently showed Maltese cross birefringence with green dichroism (Figs. 4 and 6). Green dichroism was not observed in the poorly laminated intratubular bodies in the kidneys of one of the cases of multiple myeloma. Similarly, the testicular "egg-cell-like bodies" were not dichroic and even some typical "corpora amylacea" in brain were also negative.

As shown in Figs. 4 and 6 Maltese cross birefringence and dichroism were a striking feature in pulmonary microliths and prostatic "corpora amylacea". In the prostates small fragments of dichroic material were also found in desquamated cells in the excretory ducts: this material could be interpreted as being a phagocytosed substance similar in nature to the "corpora amylacea".

No other deposits of amyloid were found in these patients.

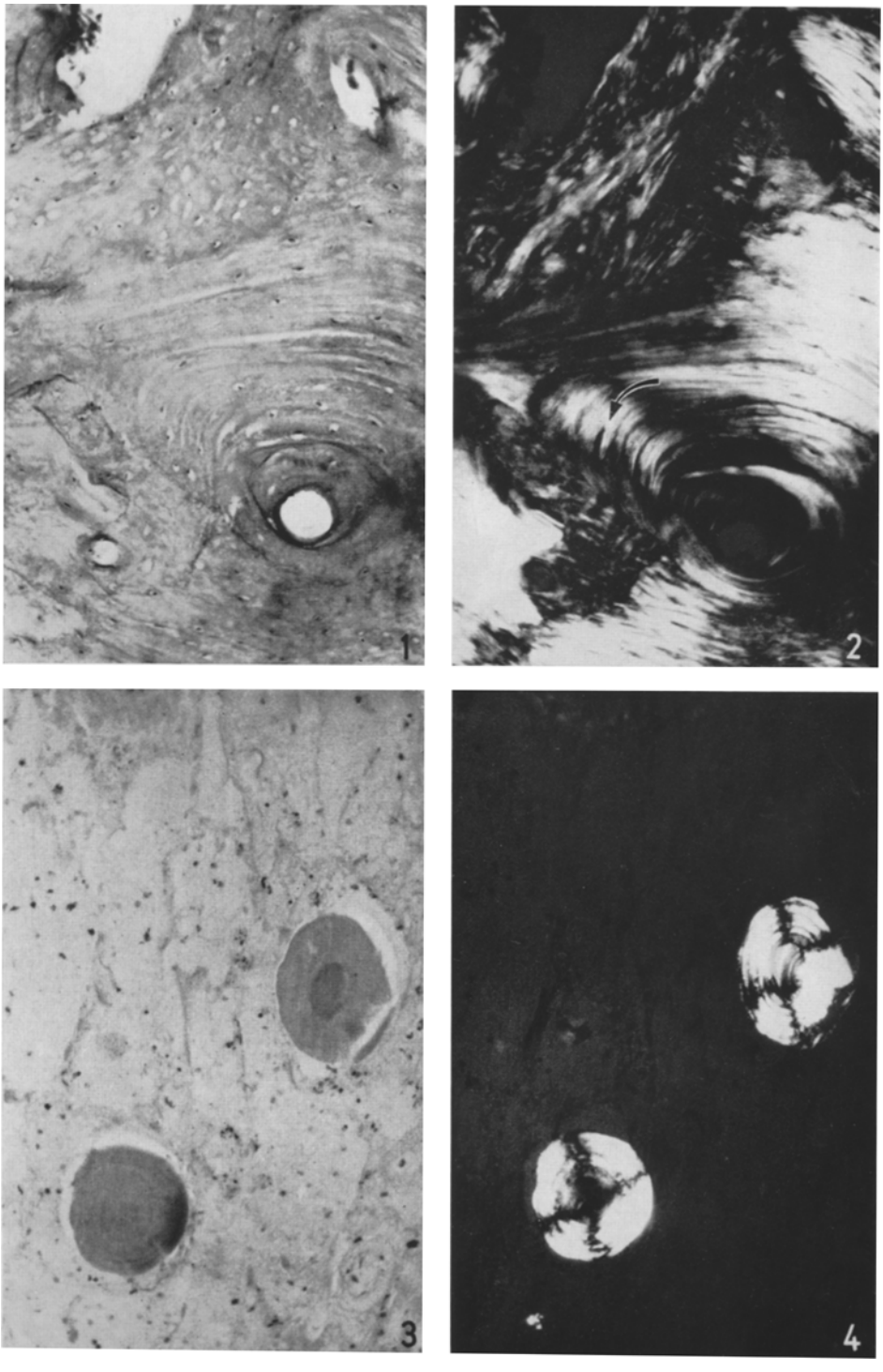

Fig. 1. Sphenoid bone of 27-year-old man who died accidentally. Congo red-haemalum staining after decalcification with trichloracetic acid. The bone was irregularly positive to Congo red. $\times$ 120

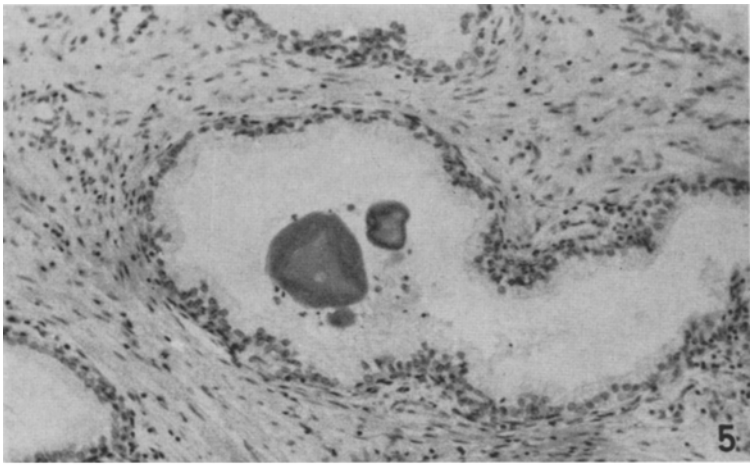

Fig. 2. Same field as Fig. 1 observed in polarized light. The Haversian system shows the typical Maltese cross, two areas of which are dichroic (arrow). × 120

Fig. 3. Section of the lung of an asthmatic patient with the socalled "pulmonary microliths" stained with Congo red and haemalum. Two heavily stained, stratified bodies are seen. × 120

Fig. 4. Same field as Fig. 3 observed in polarized light. Bright Maltese crosses with dichroic arms are seen in the "pulmonary corporea amylacea". × 120

Fig. 5. Section of a human prostatic adenoma stained with Congo red and haemalum. Two "corpora amylacea" are heavily stained with Congo red. × 120

Fig. 6. Same field as Fig. 5 observed in polarized light shows intense birefringence of the "corpora amylacea" with dichroic areas. × 120

Discussion

The molecules of Congo red are distributed in an ordered sequence in relation to the long fibril of amyloid (Puchtler *et al.*, 1962). This pattern increases the positive birefringence of amyloid and as a by-product is responsible for the green dichroism (Diezel and Pfleiderer, 1959). According to Puchtler and Sweat

(1966), the affinity of Congo red for amyloid is due to the glycoproteic nature of part of the amyloid molecule. Green dichroism in polarized light is present when there is an ordered glycoproteic molecule with affinity for Congo red. Some authors (Missmahl, 1957; Ravid *et al.*, 1967) have claimed that this property is the most sensitive and specific test for amyloid. However, there are other ordered structures very rich in glycoproteic substances which obviously do not contain amyloid material. It is known that the lamellae of bone are formed by ordered collagen fibres to which glycoproteic substances are bound (Andrews *et al.*, 1967): on examining numerous normal human and animal bones in which the presence of amyloid could be entirely ruled out we have found a green dichroic reaction, particularly in "young" areas.

"Corpora amylacea" also contain acid and neutral mucopolysaccharides, as demonstrated by their positive reaction with alcian blue-P.A.S. Their laminated structure is partly of a proteic nature. The mechanism of formation of such an ordered structure is unknown. Numerous authors have claimed an amyloid nature for "corpora amylacea" in the brain. However, we have found that some of these bodies are not dichroic. This in in agreement with Lafora's (1913) suggestion that "corpora amylacea" in the brain are of variable nature. Cerebral "corpora amylacea" are not associated only with ageing but have been reported even in very young people dying from chronic or acute diseases (Royer, 1959). All these conditions could be related to the development of amyloid deposits.

The genesis of prostatic "corpora amylacea" has been ascribed to infection and obstruction of excretory ducts. However, no other deposits of amyloid substance were found in these glands or in other organs.

Pulmonary microliths are of unknown origin and appear in the alveoli of numerous patients. Their genesis is not clear but they are generally considered to be foci of dystrophic calcification.

Even in cases of multiple myeloma, a disease in which systemic amyloidosis is not rare, the dichroic reaction of the intratubular bodies in the kidneys was positive only when an ordered structure was present.

Taking account of these observations, the occurrence of green dichroism cannot be regarded as definite proof of the amyloid nature of a substance under scrutiny.

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