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Comparisons of the Texture of Amyloid, Collagen and Alzheimer Cells

A Polarization Microscopic-Histochemical Study

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Summary. This investigation was performed in order to determine textural similarities and differences between variable amyloid types. Alzheimer fibrils and collagen were also compared with these structures. For this reason we used tissue specimens of secondary amyloid (so-called perireticular amyloid), primary amyloid (so-called pericollagen amyloid), senile plaques, Alzheimer cells and collagen of tendons, scars, atherosclerotic aortes, rheumatic synovial membranes and of a dura mater cerebri, and examined these fibre types by polarization optical-histochemical methods.

The findings allowed the conclusion that the texture of primary and secondary amyloid is not the same, because of differences in the fibrillar texture and probably of a distinctive interfibrillar substance in each case. Senile plaques seem to be a further variant of amyloid; some behaved as primary, and some as secondary amyloid. Alzheimer fibrils showed structural resemblance on senile plaques, although they contain no amyloid fibrils. The staining features of collagen clearly revealed that there is no structural relationship with amyloid, and therefore a direct transformation of collagen to amyloid and vice versa must be refuted. The collagen fibre types showed a variable texture because of their different mucopolysaccharide content. An important result is the changed hyaluronic acid metabolism of the rheumatic collagen fibres reported by other investigators.

During our investigations on amyloid, which is considered as a special case of an abnormal intercellular substance, partly equal staining-optical qualities in comparison with other fibrillar structures (e.g. collagen fibres) were observed. Thus one must ask for a structural resemblance between these elements. Reviewing literature we found references to the possibility of a relationship between the formation of collagen fibres and the formation of amyloid fibrils (Battaglia, 1966; Rodermund and Klingmüller, 1970; Schmitt and Beneke, 1971) as well as the emphatic rejection of this idea (Williams *et al.*, 1965; Lindner and Freytag, 1966). We made the attempt to compare with each other different amyloid types, senile plaques, Alzheimer fibrils and collagen fibres by means of combined polarization optical and histochemical methods. Of particular interest was the texture, i.e. complete structure consisting of fibres plus interfibrillar substance of these tissue elements. Following the experiences of polarizing-histochemical investigations reported previously (Katenkamp and Stiller, 1972) we used congo red in combination with diverse histochemical reactions.

Materials and Methods

I. Materials

For the examination we selected the following representative tissue samples:

1. Secondary amyloid (so-called perireticular amyloid). Liver, spleen, kidney and adrenal gland of 5 typical cases.

2. Senile amyloidosis of the heart as a special form of primary amyloid (so-called pericollagen amyloid).

3. Collagen fibres. 2 pyelonephritic kidneys with excessive scars, 2 atherosclerotic aortes (pars abdominalis et thoracica), 1 tendon (musculus iliopsoas), 1 dura mater cerebri, 2 synovial membranes of rheumatoid arthritis.

4. Senile plaques and Alzheimer fibrils. 2 senile brains.

Fixation in 5–10% formalin, after embedding in paraffin sections of 8 mμ thickness were produced.—Dye: congo red (p,p′-diamino-diphenyle-disazo-bi-1-naphthylamino-4-sulfonic acid) of Dr. G. Grübler &Co., Leipzig.

II. Methods of Staining

The staining with congo red took place with the modifications described by Bennhold (Bennhold-staining, see Romeis, 1968) and by Puchtler *et al.* (1962) (Puchtler-staining). The use of these methods was necessary because the mechanism of the staining reactions was different during the starting steps (Katenkamp and Stiller, 1972). The comparison methods described by Highman (1946) and Missmahl (1963) were also applied. The slices were always mounted in Canadabalsam.

III. Histochemical Methods

Before congo red staining the following histochemical blocking, oxydizing and substitution methods were performed: periodic acid oxydation, periodic acid oxydation and following amineraldehyde condensation, acetylation, acetylation and reverse the acetylation process by dilute alkali (0.1 N KOH), mild and strong methylation, methylation followed by saponification to remove methyl esters, controlling this effect the sections were only treated with alcoholic KOH, treatment with sulfuric acid, deamination (Lillie), oxydation with ninhydrine, chloramine-T-reaction, blocking of SH groups, blocking of SH groups and reduction of disulfide groups by means of KCN to active SH-groups, bromsuccinimide oxydation, treatment with acroleine, benzoylation, treatment with dinitrofluorobenzole (DNFB), reaction with dimethylaminobenzaldehyde (Adams, 1957), treatment with concentrated HCl for 2 min corresponding with the conditions of Adam's reaction, and coupled tetrazonium reaction.

Taking into account the results of former experiments a coupled tetrazonium reaction was interposed between the treatment with KOH or HCl and the staining with congo red. Moreover a digestion with hyaluronidase was performed (methods of the reactions see Burstone, 1959; Spannhof, 1967).

IV. Evaluation of the Results

Beside the usual light microscopical evaluation we examined the congo red stained sections by means of the polmi-equipment of the Universalforschungsmikroskop NU of VEB Carl Zeiss Jena in polarized ordinary light. The following was tested:

1. The optical sign of birefringence,
2. the dye dichroism and
3. the existence of anomalous green polarization colour.

The sections were prepared under equal conditions and therefore the results were comparable and were classified as follows: ∅ = white to yellowish colour, + = moderate and ++ = strong intensity of green colour.

In order to prove the findings reiterations were performed with new solutions and both investigators separately evaluated the staining results.

V. Additional Staining Methods

H. E., Elastica-van Gieson, alcianblue-PAS, alcianblue pH 1.0 and pH 2.5 (Spicer and Henson, 1967), thioflavine S (Schwartz, 1965) and thioflavine T (Burns *et al.*, 1967).

Results

Staining Results

Amyloid of primary and secondary amyloidosis, senile plaques and Alzheimer fibrils were stained clearly red by congo red. Dense-textured collagen fibres (larger scar areas, tendon, dura mater cerebri) also showed red colour with Bennhold's procedure. In general, a diminishing of the staining intensity in conformity with the used modifications can be observed. The Bennhold-method presents the strongest staining effect, the Missmahl-modification the lowest one.

Polarization Microscopy

Not all congo red positive structures appear in green polarization colour between crossed polaroids. Only amyloid, senile plaques and Alzheimer fibrils are green positive after staining with the Puchtler-method; collagen fibres always show a white to yellowish birefringence. However, some collagen fibres are green between crossed polaroids after Bennhold-modification, chiefly fibres with dense texture. This result is virtually caused by the non-standardized time of differentiation (Katenkamp and Stiller, 1972).

The examination of structures with green polarization colour showed a red-colourless dichroism and a positive birefringence. This behaviour is typical for amyloid (compare Missmahl, 1966 and 1968). The remaining red stained tissue elements only showed a positive birefringence (positive intrinsic and form birefringence of collagen fibres!); dichroitic phenomena were always absent.

The Effect of Histochemical Pretreatments on Dye Arrangement

The findings show different results of the separate structures in the cases of amyloid, collagen and senile brain alterations. They show an only partial conformity of closely related structures as in cases of different types of amyloid and also collagen fibres (Table 1). The different polarization optical findings can generally be explained by variable arrangement of the dye molecules. A detailed analysis of the red colour in the light microscope after the substitution and blocking methods was not performed because an important contribution to the structural comparison was not to be expected.

Puchtler and Bennhold-modifications differ widely from each other after polarization optical evaluation: The former shows differences between bot amyloid types after bromsuccinimide reaction and treatment with alkali. Although the reversing of the acetylation process by dilute alkali and saponification after methylation induce clear effects, these results must be explained as consequences of alkali treatment. The behaviour of the collagen fibre group is chiefly uniform, only the collagen fibres of the rheumatoid synovial membrane differ from the rest after blocking of SH groups and treatment with hyaluronidase. The differences between senile plaques and Alzheimer fibrils are obviously caused by extraction effects (KOH, HCl).

Table 1. Polarization optical-histochemical results related to the intensitiy of the anomalous green polarization colour. x = Collagen from Dura mater, scars, tendon etc., xx = Fine collagen fibres from rheumatoid synovial membrane

Reactions	Bennhold-staining						Puchtler-staining					
	Amyloid		Collagen		Senile plaque	Alz-heimer cells	Amyloid		Collagen		Senile plaque	Alz-heimer cells
	prim.	second.	x	xx			prim.	second.	x	xx		
Untreated	+/++	+/++	ø/+	ø	+/++	+/++	++	++	ø	ø	++	++
Periodic acid oxydation	+/++	+/++	ø/+	ø	+/++	+/++	++	++	ø	ø	++	++
Periodic acid oxydation and amine-aldehyde condensation	ø	ø/+	ø/+	ø	ø	ø	+/++	++	ø	ø	++	++
Acetylation	ø	ø	ø	ø	ø	ø/+	+/++	+/++	ø	ø	+/++	+/++
Acetylation and reverse by alkali	ø	ø	ø	ø	ø	ø	+	ø	ø	ø	ø	ø
Methylation mild	+/++	+/++	ø/+	ø/+	+	+	++	++	ø	ø	++	++
strong	+/++	+/++	ø/+	ø/+	+/++	+/++	++	+/++	ø/+	+	+/++	+/++
Methylation and following saponification	+	ø	ø	ø	ø	ø	+/++	ø	ø	ø	+	+/++
Alcoholic KOH	ø/+	ø	ø/+	ø	ø	ø	ø	ø	ø	ø	+	++
Sulfuric acid treatment	ø	ø	ø	ø	ø	ø	+	+/++	ø	ø	+	+/++
Deamination	ø	ø	ø/+	ø	ø	ø/+	++	++	ø	ø	++	++
Ninhydride	+/++	+/++	+	+	+/++	+/++	+/++	+/++	ø	ø	+/++	+/++
Chloramine T	ø	ø	ø	ø	ø	ø	+/++	++	ø	ø	++	++

Blocking of SH groups	∅/+	+/++	+	+	+/++	+/++	++	++	∅	+	++	++
Blocking of SH groups and KCN	∅/+	+/++	∅	∅/+	+	∅/+	++	++	∅	∅/+	++	++
Bromsuccinimide	∅	+/++	∅	∅	∅	∅	+/++	++	∅	∅	++	++
Acroleine	∅/+	+/++	∅	∅	∅/+	∅	++	++	∅	∅	++	++
Benzoylation	∅/+	∅/+	∅	∅	∅/+	∅	+/++	+/++	∅	∅	+	∅
DNFB	+	+/++	∅	∅	∅/+	∅/+	++	++	∅	∅	++	++
Adam's reaction	++	+/++	∅/+	∅	+/++	+/++	∅	∅	∅	∅	∅	+/++
HCl-extraction	+/++	+/++	∅/+	∅	+/++	+/++	∅	∅	∅	∅	∅	+
Coupled tetrazonium salt reaction	+	+/++	+/++	+/++	+/++	+/++	++	++	+/++	+/++	+/++	++
KOH-coupled tetrazonium salt reaction	+	+/++	+/++	+/++	/	/	+/++	+/++	+/++	+/++	/	/
HCl-coupled tetrazonium salt reaction	+	+	+/++	+/++	/	/	+/++	+/++	+/++	+	/	/
Hyaluronidase	+	+/++	∅	∅/+	∅	+/++	++	++	∅	+	++	++

The findings after Bennhold-modification are somewhat different. Only KOH produces a strong extraction effect on secondary amyloid, pretreatment with acid is not efficient. Alterations of the SH-groups including treatment with bromsuccinimide, acroleine and DNFB only change the dye arrangement on primary amyloid. It is difficult to survey the behaviour of collagen fibres (compare periodic acid oxydation, Adam's aldehyde condensation, extraction with HCl and deamination). The result after hyaluronidase digestion of Alzheimer fibrils in comparison to senile plaques is of particular interest.

Moreover it can be pointed out that after coupled tetrazonium reaction all investigated fibre structures show a green colour in the polarization microscope between crossed polaroids in spite of interposing an HCl or KOH extraction. The complete results are summarized in Table 1.

Additional Staining Methods

These staining methods should provide the possibility of a definite localization of amyloid and informations about the content of mucopolysaccharides. In the case of amyloid various findings resulted: in larger deposits there were predominantly PAS-positive central areas, whereas peripheral areas were stained with alcianblue. In the heart, however, in case of primary amyloid exclusive alcianblue-positive fibre-like deposits were found (Stiller and Katenkamp, 1971). Thicker bundles of collagen fibres mainly exhibited positive PAS-reaction. Many thin collagen fibrils in the synovial membrane were also alcianblue-positive. Alzheimer fibrils and senile plaques mostly presented a positive PAS-reaction, an alcianophilic reaction was almost completely absent; but in some senile plaques a faint blue colour can be observed when this method (pH 2.5) is used.

Discussion

The anomalous green polarization colour after congo red staining and the dichroism indicate that the linear dye molecules are arranged in parallel on amyloid, senile plaques and Alzheimer fibrils. This is also valid for a little part of collagen fibres as evidenced by an identical behaviour after Bennhold-staining. Therefore a textural resemblance of all structures with a green polarization colour could be suggested. We found, however, differences using detailed polarization optical-histochemical investigations.

For their interpretation one must bear in mind the nature of dye arrangement. In an investigation on amyloid we previously demonstrated that the dye micelles are bound nonionic-complex on amyloid fibrils in case of the Puchtler-modification. For this reason the particular importance of the strong fibrillar dipole moment and the correspondingly oriented structure of the perifibrillar space must be emphasized. The coplanarity between dye molecule and amyloid fibril postulated by others appears to be not so significant. Moreover, the Bennhold-method distinguishes by an initial accumulation of dye molecules bound by NH_2 and OH groups of the substrate in salt-like manner (Katenkamp and Stiller, 1972). It is impossible to characterize special side groups with these methods, because in apolar hydrophobic mounting media (as Canadabalsam), in which neither dye molecules nor fibrils possess electric charges, congo red molecules

are bound by intermolecular forces and hydrogen bondings. For that reason both methods allow hints at possible textural resemblances resp. differences. Thus only comparisons between the analyzed structures are permitted.

The samples of reactions of amyloid are generally interpreted as different compositions of secondary and primary amyloid. We explain the behaviour of Puchtler-staining after KOH and HCl pretreatment as extraction and following masking effects. A partial extraction of interfibrillar substance is supposed presuming a template function of non-altered fibrils for restitution of polarization optical phenomena after coupled tetrazonium reaction. Additionally we must point out that secondary amyloid is more sensitive against KOH treatment. Corresponding to the models reported by Scheuner *et al.* (1971) a directed fibrillar-oriented arrangement of these interfibrillar macromolecules has to be assumed. Many investigators have emphasized the different contents of mucopolysaccharides in amyloidotic substances (Clausen and Christensen, 1964; Battaglia and Matturri, 1965; Berenson *et al.*, 1967). Although even highly purified amyloid fibrils have an anomalous green polarization colour after staining with congo red (see Glenner *et al.*, 1972) we believe that the arrangement of mucopolysaccharides is of importance for the parallel aligment of congo red micelles in amyloidotic tissue specimens.

Concerning the behaviour of the Bennhold-staining after KOH treatment and following coupled tetrazonium reaction we also conclude that a part of the interfibrillar substance was resolved followed by destruction of the ordinary texture. The results support the view, that the separate amyloid types have different substances coating the fibrils.

Using the Bennhold-modification alterations of SH groups (blocking of SH groups, acroleine and bromsuccinimide reaction) cannot result in influencing the salt-like bindings between congo red molecules and amyloid fibrils since salt-like binding is impossible between the acid dye molecule and SH groups in a watery medium. Their effects were conceivable in consequence of ionic changes with following alteration of perifibrillar texture as well as of masking effects.

A similarity of amyloid fibrils was supposed by reason of applying chemical and physical methods (Gueft *et al.*, 1968; Bonar *et al.*, 1969). On the other hand reports are published, which contain informations about differing amyloid fibrils in different individuals (Glenner *et al.*, 1970, 1971; Benditt and Eriksen, 1971; Ein *et al.*, 1972). But informations about amino acid analyses should be used carefully in view of the difficulties in isolating highly purified fibrils (Pras *et al.*, 1969). The simulation of a differing amino acid content by different contaminations and enzymatic processes must also be kept in mind. Moreover Miller *et al.* (1968) emphasized the influence of isolation methods. It was not the goal of our investigation, however, to clarify this disputed question.

Collagen fibres and amyloid types differ from each other by polarization optial effects in the Puchtler-stained formol-fixed samples. Strong methylation and coupled tetrazonium reaction alter the collagen. Now the dye micelles are bound in nearly axiparallel order to the surface of collagen fibres (as on amyloid fibrils). In the former case as in the case of amyloid the arrangement of mucopolysaccharides and their alteration by eliminating of acid groups are of great importance. Quite unexpected results by Bennhold-staining after elimination

of amino groups, periodic acid oxydation and after HCl treatment can only be explained by structural alterations. These results strengthen the assumption that a textural resemblance between amyloid and collagen is not existent. In this connexion one should refer to Romhanyi's findings, too (Romhanyi, 1970 and 1971).

Comparing mature collagen fibres with fibres of rheumatoid synovial membrane attention had to be called to the result after digestion with hyaluronidase. The results can be related to the findings of newer experiments by Castor *et al.* (1971), which proved an altered metabolism of hyaluronic acid in cell cultures of rheumatoid synovial membrane.

The senile plaques show a sample of reactions resembling amyloid. This is a further evidence for the amyloid nature of these structures, which already was found earlier (Terry *et al.*, 1964; Katenkamp and Stiller, 1971; Nikaido *et al.*, 1971). A point worth stressing is that the perifibrillar substance of senile plaques somewhat varies from primary as well as secondary amyloid, as evidenced by histochemical methods and digestion with hyaluronidase. Furthermore it is remarkable, that senile plaques are able to behave as primary or secondary amyloid.

The fibrils in Alzheimer cells are certainly of other nature, confirmed by ultrastructural investigations (Luse and Smith, 1964; Terry *et al.*, 1964; Schlote, 1968). Therefore the texture of neurofibrillary tangles differs from amyloid after some histochemical reactions.

We can draw the following conclusions from our results:

1. A distinguishing characteristic of the amyloid types is the different texture. From this viewpoint it must be concluded, that amyloid is a heterogenous substance (compare Benditt *et al.*, 1971; Benditt and Eriksen, 1972; Romhanyi, 1972). Primary amyloid, secondary amyloid and senile plaques are identical per se in each case.

2. Generally the textures of amyloid and collagen differ from each other fundamentally. The possibility of a direct transformation of collagen to amyloid and vice versa must be refuted.

3. Collagen posseses a varying texture. Probably an altered content of mucopolysaccharides is of primary importance.

4. Obviously the texture of neurofibrillary tangles is not identical with amyloid, but some textural resemblances can be found, too.

References

Adams, C. W. M.: A p-dimethylaminobenzaldehyde nitrite method for the histochemical demonstration of tryptophane and related compounds. J. clin. Path. **10**, 56–62 (1957).

Battaglia, S.: Contribution to Caesar's report: Elektronenmikroskopische Befunde am Amyloid. In: Fortschritte der Amyloidforschung, hrsg. von G. Bruns, W. Zschiesche und S. Fritsch, Nova Acta Leopoldina **31**, 101–102 (1966).

Battaglia, S., Matturri, L.: Mucoplysaccharides in amyloidosis. Lancet **1965 I**, 1282–1283.

Benditt, E. P., Eriksen, N.: Chemical classes of amyloid substance. Amer. J. Path. **65**, 231–252 (1971).

Benditt, E. P., Eriksen, N.: Chemical characteristics of the substance of typical amyloidosis in monkeys. Acta path. microbiol. scand. Section A 80, Suppl. **233**, 103–108 (1972).

Benditt, E. P., Eriksen, N., Hermodson, M. A., Ericsson, L. H.: The major proteins of human and monkey amyloid substance: common properties including unusual N-terminal amino acid sequences. FEBS Letters **19**, 169–173 (1971).

Berenson, G. S., Dalferes, E., Radhakrishnamurthy, B.: Glycosaminoglycans in experimental amyloidosis. Fed. Proc. **26**, 300 (1967).

Bonar, L., Cohen, A. S., Skinner, M. M.: Characterization of the amyloid fibril as a cross-beta protein. Proc. Soc. exp. Biol. (N.Y.) **131**, 1373–1375 (1969).

Burns, J., Pennock, C. A., Stoward, P. J.: The specifity of the staining of amyloid deposits with thioflavine T. J. Path. Bact. **94**, 337–344 (1967).

Burstone, M. S.: Histochemical methods for protein detection. In: Handbuch der Histochemie, hrsg. von W. Graumann und K. Neumann. Stuttgart: G. Fischer 1959.

Castor, C. W., Dorstewitz, E. L., Rowe, K., Ritchie, J. C.: Abnormalities of connective tissue cells cultured from patients with rheumatoid arthritis. II. Defective regulation of hyaluronate and collagen formation. J. Lab. clin. Med. **77**, 65–75 (1971).

Clausen, J., Christensen, H. E.: Paraproteins and acid mucopolysaccharides in primary amyloidosis. Acta path. microbiol. scand. **60**, 493–511 (1964).

Ein, D., Kimura, S., Glenner, G. G.: An amyloid fibril protein of unknown origin: partial amino-acid sequence analysis. Biochem. biophys. Res. Commun. **46**, 498–500 (1972).

Glenner, G. G., Ein, D., Terry, W. D.: The immunglobulin origin of amyloid. Amer. J. Med. **52**, 141–146 (1972).

Glenner, G. G., Harada, M., Isersky, C., Cuatrecasas, P., Page, D., Keiser, H.: Human amyloid protein: diversity and uniformity. Biochem. biophys. Res. Commun. **41**, 1013–1019 (1970).

Glenner, G. G., Page, D., Isersky, C., Harada, M., Cuatrecasas, P., Eanes, E. D.: Murine amyloid fibril protein: isolation, purification and characterization. J. Histochem. Cytochem. **19**, 16–28 (1971).

Gueft, B., Kikkawa, Y., Hirschl, S.: An electron-microscopic study of amyloidosis from different species. In: Proceedings of the Symposium on Amyloidosis, ed. by E. Mandema, L. Ruinen, J. H. Scholten and A. S. Cohen, p. 172–182. Amsterdam: Excerpta Medica 1968.

Highman, B.: Improved methods for demonstrating amyloid in paraffin section. Arch. Path. **41**, 559–565 (1946).

Katenkamp, D., Stiller, D.: Der Alzheimer'sche Symptomenkomplex (kongophile Angiopathie, senile Plaques und Alzheimersche Fibrillenveränderungen) und seine Beziehung zum Amyloid. Zbl. allg. Path. path. Anat. **114**, 409–417 (1971).

Katenkamp, D., Stiller, D.: Polarisationsoptisch-histochemische Untersuchungen zur Kongorotfärbung des Amyloid. Histochemie **29**, 37–43 (1972).

Lindner, J., Freytag, G.: Zur Amyloidentstehung. In: Fortschritte der Amyloidforschung, hrsg. von G. Bruns, W. Zschiesche and S. Fritsch. Nova Acta Leopoldina **31**, 131–141 (1966).

Luse, S. A., Smith, K. R.: The ultrastructure of senile plaques. Amer. J. Path. **44**, 553–563 (1964).

Miller, H. J., Rotman, Y., Ben-Shaul, Y., Ashkenazi, Y.: The dissoziation of the amyloid filament to subunits. Israel J. med. Sci. **4**, 982–986 (1968).

Missmahl, H. P.: Rectumbiopsie zum Nachweis der Amyloidose. Dtsch. med. Wschr. **88**, 1783–1785 (1963).

Missmahl, H. P.: Polarisationsoptische Befunde am Amyloid. In: Fortschritte der Amyloidforschung, hrsg. von G. Bruns, W. Zschiesche and S. Fritsch. Nova Acta Leopoldina **31**, 79–85 (1966).

Missmahl, H. P.: Reticulin and collagen as important factor for the localization of amyloid. The use of polarization microscopy as a tool in the detection of the composition of amyloid. In: Proceedings of the Symposium on Amyloidosis, ed. by E. Mandema, L. Ruinen, J. H. Scholten and A. S. Cohen, p. 22–29. Amsterdam: Excerpta Medica 1968.

Nikaido, T., Austin, J., Rinehart, R., Trueb, L., Hutchinson, J., Stukenbrok, H., Miles, B.: Studies in ageing of the brain. I. Isolation and preliminary characterization of Alzheimer plaques and cores. Arch. Neurol. **25**, 198–211 (1971).

Pras, M., Zucker-Franklin, D., Rimon, A., Franklin, E. C.: Physical, chemical, and ultrastructural studies of water-soluble human amyloid fibrils. J. exp. Med. **130**, 777–795 (1969).

Puchtler, H., Sweat, F., Levine, M.: On the binding of congo red by amyloid. J. Histochem. Cytochem. **10**, 355–364 (1962).

Rodermund, O. E., Klingmüller, G.: Zur submikroskopischen Struktur des Amyloid. Arch. klin. exp. Derm. **236**, 147–160 (1970).

Romeis, B.: Mikroskopische Technik. München-Wien: R. Oldenburg 1968.

Romhanyi, G.: Selective differentiation between amyloid and connective tissue structures based on the collagen specific topo-optical staining reaction with congo red. Virchows Arch. Abt. A **354**, 209–222 (1971).

Romhanyi, G.: Differences in ultrastructural organization of amyloid as revealed by sensitivity or resistance to induced proteolysis. Virchows Arch. Abt. A **357**, 29–52 (1972).

Romhanyi, G., Deák, G. Bukovinszky, A.: On the collagen-specific topo-optical staining reaction with congo red and its ultrastructural interpretation. Acta morph. Acad. Sci. hung. **18**, 261–282 (1970).

Scheuner, G., Hutschenreiter, J., Franz, H.: Modelle zur Erklärung des Zusammenhanges zwischen Metachromasie, Doppelbrechung und künstlichem Dichroismus an biologischen Objekten. Acta histochem. (Jena) **40**, 23–28 (1971).

Schlote, W.: Polarisationsoptisch differenzierbare Stadien der intraneuronalen Amyloidbildung bei Morbus Alzheimer. Verh. dtsch. Ges. Path. **52**, 204–209 (1968).

Schmitt, W., Beneke, G.: Die Amyloidbildung als Ausdruck einer altersbedingten Änderung der Syntheseleistung von Bindegewebszellen. Akt. Gerontol. **1**, 649–652 (1971).

Schwartz, P.: Über Amyloidose des Gehirns, der Langerhansschen Inseln und des Herzens alter Personen. Zbl. allg. Path. path. Anat. **108**, 169–176 (1965).

Spannhof, L.: Einführung in die Praxis der Histochemie. Stuttgart: G. Fischer 1967.

Spicer, S. S., Henson, G. G.: Methods for localizing mucosubstances in epithelial and connective tissues. Meth. Achiev. exp. Path. **2**, 78–91 (1967).

Stiller, D., Katenkamp, D.: Zur Pathogenese der senilen Amyloidose. Virchows Arch. Abt. A **352**, 209–218 (1971).

Terry, R. D., Gonatas, N. K., Weiss, M.: Ultrastructural studies in Alzheimer's presenile dementia. Amer. J. Path. **44**, 269–297 (1964).

Williams, G., Gowenlock, A. H., Wynn, C.: Studies of collagen synthesis in experimental amyloidosis. Brit. J. exp. Path. **46**, 408–412 (1965).

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