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DIFFERENCE IN POTASSIUM PERMANGANATE SENSITIVITY BETWEEN PRIMARY AND SECONDARY AMYLOIDOSIS

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Summary

The permanganate method was applied to tissues from **35** cases of amyloidosis seen at the University Hospital. Kuala Lumpur. This method is based on the difference in affinity of various chemical types of amyloid for Congo red dye after treatment with potassium permanganate. Our findings agree with the concept that amyloid of largely immunoglobulin light chain composition (primary and myeloma amyloid) is permanganate-resistant while amyloid composed predominantly of AA protein (secondary amyloid) is sensitive. This simple histochemical method is a useful laboratory aid in the classification o famyloidosis.

INTRODUCTION

While amyloid continues to be defined by its histochemical, X–ray diffraction and electron microscopical features, the biochemical nature of the amyloid fibril is known to differ in various forms of amyloidosis.' The fibrillar protein of primary and myeloma amyloid (AL protein) has been demonstrated to contain peptide units identical to the amino-terminal variable regions of immunoglobulin light chains. On the other hand, the secondary amyloid fibril is largely composed of the AA protein which does not resemble any immunoglobulin. Although subtyping of the amyloid proteins will richly contribute to our understanding of amyloidogenesis, it is no easy task. Isolation and purification of the fibril protein from amyloid deposits to determine its biochemical nature is clearly not within the ability of most laboratories. No reliable conclusions have been drawn from the type of organ involvement or the histological patterns of amyloid deposition. In 1977, Wright et *al*[2] demonstrated a difference in affinity of various chemical types of amyloid for the Congo red dye after treatment with potassium permanganate. We have applied this histochemical method to tissues containing amyloid to assess its usefulness in differentiating primary from secondary amyloid.

MATERIALS AND METHODS

The ***procedure*** applied to formalin-fixed paraffin-embedded 5pm sections of tissues containing amyloid is as follows:

1. Deparaffinise in xylol and rehydrate through alcohol to distilled water.
2. Treat with acidified potassium permanganate (equal parts of **0.3%** H_2SO_4 and **0.5%** $KMnO_4$) for **5** minutes.
3. Bleach in **2%** oxalic acid.
4. Wash in running water.
5. Stain with Harris's haematoxylin for **5** minutes.
6. Wash in running water.
7. Immerse in alkaline **80%** ethanol saturated with sodium chloride for **20** minutes.
8. Stain with alkaline alcoholic saturated Congo red for 20 minutes.
9. Dehydrate in **2** changes of absolute alcohol.
10. Clear in **2** changes of xylene.
11. Mount in protexx.

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The *tissues examined* had all been shown earlier to contain amyloid deposits through Congo red positivity, metachromasia with crystal violet and fluorescence with the thioflavine-T stain. Electron microscopy was performed on a large number of specimens (21/35) and this demonstrated the typical non-branching fibrils of amyloid. These tissues were obtained from 35 subjects with amyloidosis, seen at the University Hospital, Kuala Lumpur. Six of them had generalised amyloidosis for which no cause was found (clinical primary amyloidosis) while one had multiple myeloma with amyloid casts in the renal collecting ducts. The remaining 28 subjects were believed to be suffering from secondary amyloidosis: 7 had tuberculosis,[3] 2 had leprosy and 19 had chronic heart disease associated with amyloidosis of the heart.[4]

RESULTS

A specimen was considered permanganate-sensitive if all or more than 80% of the amyloid deposits failed to stain with Congo red after treatment with potassium permanganate. As depicted in Table 1, we found that myeloma associated amyloid and the deposits of 4 out of 6 cases of primary amyloidosis were resistant to potassium permanganate. Secondary amyloid was uniformly permanganate sensitive (Figs. 1 a & b).

DISCUSSION

Our findings are in agreement with those of Wright *et al.* Secondary amyloid which is composea predominantly of the AA protein was permanganate-sensitive while amyloid of largely immunoylobulin light chain composition was usually resistant. The 2 cases of clinical primary amyloidosis with permanganate sensitive deposits were Orang Asli subjects.[3] As the rate of infections among the Malaysian aborigines is known to be high,[5] it is very likely that both of these subjects had secondary amyloidosis in which the underlying disease was overlooked. Thus, the histochemical method applied here can be considered relatively reliable in differentiating primary from secondary amyloidosis. It is simple to perform and is not time consuming. It does not require fastidious treatment of specimens, can be applied to formalin-fixed, paraffin-embedded tissue and is therefore useful even in retrospective studies. The application of this method in our histopathology laboratories will be useful in the classification of amyloidosis and may one day prove helpful in selecting patients for treatment.

The mechanism of action of potassium permanganate on AA protein is as yet unknown. it is believed that loss of Congo red affinity probably results from alteration of the B-pleated sheet configuration of amyloid.

TABLE 1
POTASSIUM PERMANGANATE REACTION IN 35 CASES OF AMYLOIDOSIS

Clinical type of Amyloidosis	No. of cases	$KMnO_4$ Reaction	
		No. Resistant	No. Sensitive
1. Primary	6	4	2
2. Myeloma	1	1	0
3. Secondary			
Tuberculosis	7	0	7
Leprosy	2	0	2
Chronic heart disease	19	0	19

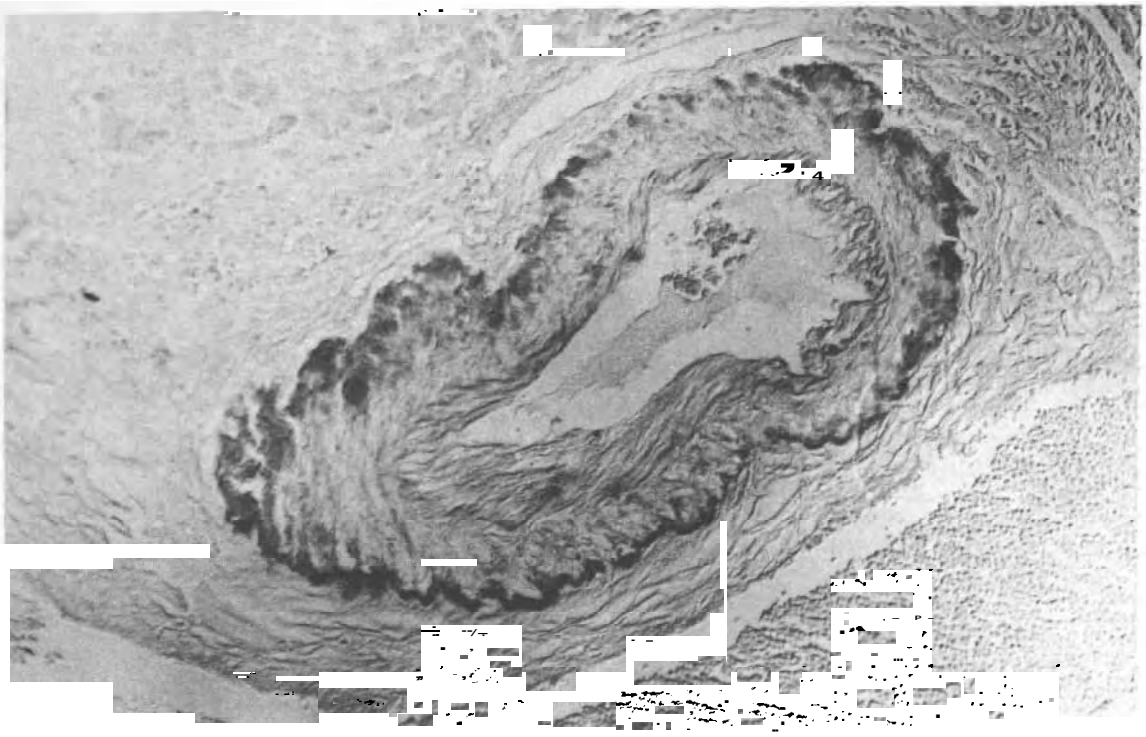

Figure 1a. *A medium-sized hepatic artery from a patient with secondary amyloidosis. Congo red positive amyloid deposits are present in the adventitia of the vessel and appear dark-grey in this photomicrograph. Congo red x 150.*

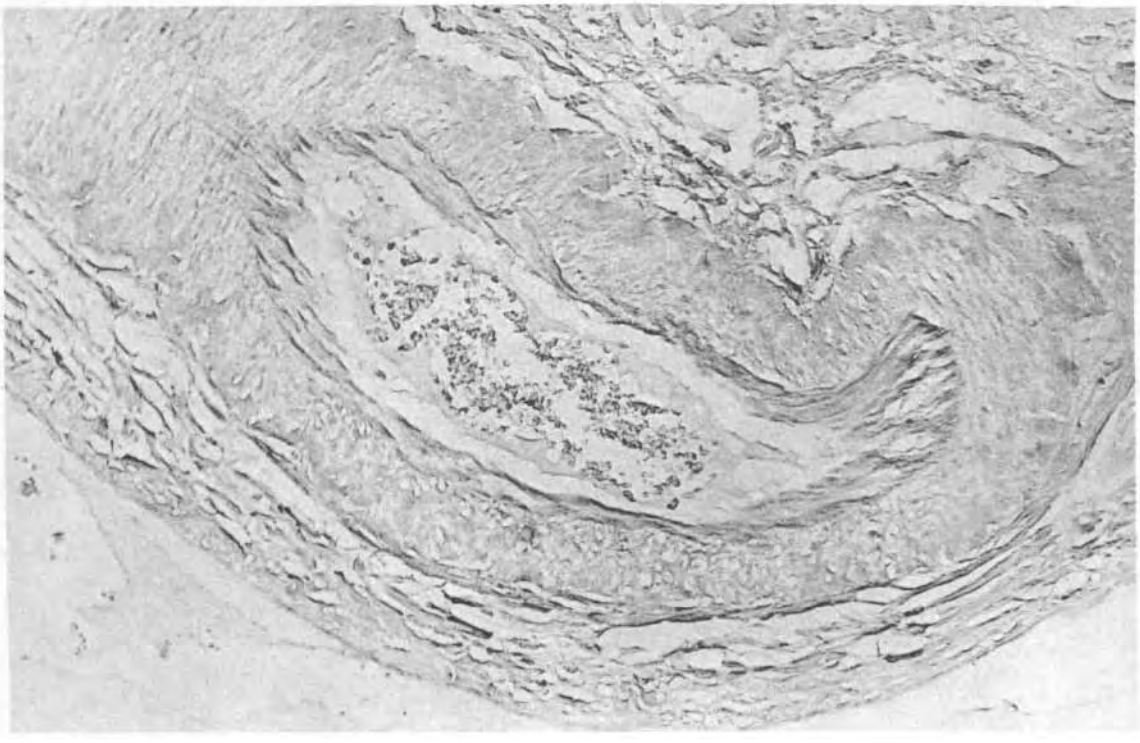

Figure 1b. *A deeper section of the same artery as in 1a, stained with Congo red after treatment with potassium permanganate. The amyloid deposits are no longer Congo red positive. Congo red – $KMnO_4$ x 150.*

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