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Lipofuscin and Transsynaptic Degeneration

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Summary. The periodic acid Schiff reaction was applied to neurones in the Lateral Geniculate Body (L.G.B.) of a series of normal and blind patients over a wide age range. The quantity of the reaction product was determined as a measure of lipofuscin which was found to increase linearly with age in the L.G.B. neurones. The amount in the neurones of the blind patients was found to be less than that in the controls, and a further group of four patients with apparently normal vision and central nervous system disease had an intermediate amount of lipofuscin. It is suggested that the component of lipofuscin measured by this technique represents an index of both metabolic activity and functional activity of the neurone. The reduced amounts in the patients with C.N.S. disease may reflect reduced visual function in association with non-visual pathway disease.

Key words: Lipofuscin – Periodic acid Schiff – Blindness.

Oxidative enzymes have been used as a measure of transsynaptic degeneration of the L.G.B. in experimental animals (Cotlier et al., 1965) and have been found in reduced quantities where there are lesions in the optic tracts. Enzymes have also been shown to reflect transsynaptic degeneration earlier than loss of nissl substance (Gay et al., 1964). Thus a study of lipofuscin, an oxidative product, will provide information on the activity of oxidative enzymes in autopsy material when the enzymes have undergone autolysis.

Lipofuscins are insoluble lipids at varying stages of oxidation, resistant to alcoholic dehydration and paraffin embedding and are eminently suitable for examination in autopsy material. Since their histochemical characteristics vary as oxidation continues any one technique will not demonstrate the total amount of lipofuscin in the tissue. Here we have quantitated that component of lipofuscin with 1, 2-glycol groups and used this as a measure of the metabolic activity of the neurone. The periodic acid Schiff reaction has been used to measure glycogen in polymorphs (Gahrton, 1964), but has not been used quantitatively

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in tissue sections. Since the intensity of the colour developed is in some way related to the amount of stainable material present this technique may be applicable in cytophotomorphometry using tissue sections.

Lipofuscin accumulates linearly with age in the human nervous system (Samorajski et al., 1964) and in animals, where the amount has been shown to vary with the cell type (Reichel et al., 1968). The aims of this paper are to demonstrate that the amount of dye formed by the P.A.S. reaction in tissue sections is proportional to the section thickness and to apply this technique to the neurones in the L.G.B. of a series of brains from normal and blind patients over a wide age range. We wished to determine whether lipofuscin accumulates at this site with age, and to use these results as controls for the blind patients.

Materials and Methods

Paraffin blocks of corpus callosum were prepared from material fixed in 10% neutral formalin for 10 days obtained at autopsy in a 20 year old woman who had died of non-neurological disease. Sections were cut at one micron intervals from 5–20 μ and attached to glass slides without adhesive. Simultaneous staining of these was carried out using the alcoholic P.A.S. reaction of Hotchkiss (1948) using Schiff's reagent (Tomasi, 1936) and sulphite rinses (Feulgen and Rossenbeck, 1924) to prevent any Schiff reagent in the tissue being oxidised to give a spurious colour reaction (Pearse, 1972). Counter stains were omitted. Microspectrophotometric determinations were carried out using a Vickers M85 Microdensitometer from 400–700 nm (Fig. 1) using the 8 μ section. The extinciton maxima is 540 nm, similar to that obtained by other authors (Kasten, 1960). Although slight variation of this peak occurs with different batches of Basic Fuchsin (Kasten, 1960) only one batch was used during these experiments. To stabilise the SO_2 level in the schiff reagent, it was prepared in one batch, transferred to small tightly stoppered bottles and stored at 4° C.

Readings of extinction were made at 540 nm in all sections and plotted against the corrected section thickness (Fig. 2) obtained using interference microscopy (Baker, 1957). (Coefficient of correlation 0.94) These results show that there is a linear relationship between the amount of stainable material and the colour density, and that the P.A.S. reaction obeys Beer's law under these conditions at the section thickness used. This linear relationship has been found to apply to the colour developed in solution (Scott and Harbinson, 1971) when the substitute is a polyaldehyde rather than a monoaldehyde suggesting the reactive component in lipofuscin is a polyaldehyde.

Brains from 34 patients from 5 days to 85 years were obtained at autopsy and fixed in 10% neutral formalin for 10–14 days; 18 were normal with no evidence of neurological disease, 12 had had visual deprivation for periods of one to 20 years, and 4 were apparently visually normal but 2 of these cases were totally deaf and a further 2 had clinical and morbid anatomic evidence of Alzheimer's disease.

The paraffin sections were prepared from each L.G.B. to include all 6 layers and stained with P.A.S. as described. The extinction of 30 cells from layers 5 and 6 on each side was determined using the Vickers integrating microdensitometer and a mean calculated.

Results

In normal cases lipofuscin increased linearly with age (Fig. 3). However, in those instances where the patients were blind there was marked reduction in the amount of lipofuscin present. In one instance where the patient had had one eye enucleated the difference between the quantity of lipofuscin in layers 6 (blind) and 5 (normal) is microscopically obvious (Fig. 4). In those cases where there was organic brain disease or deafness intermediate values for the amount of lipofuscin were obtained.

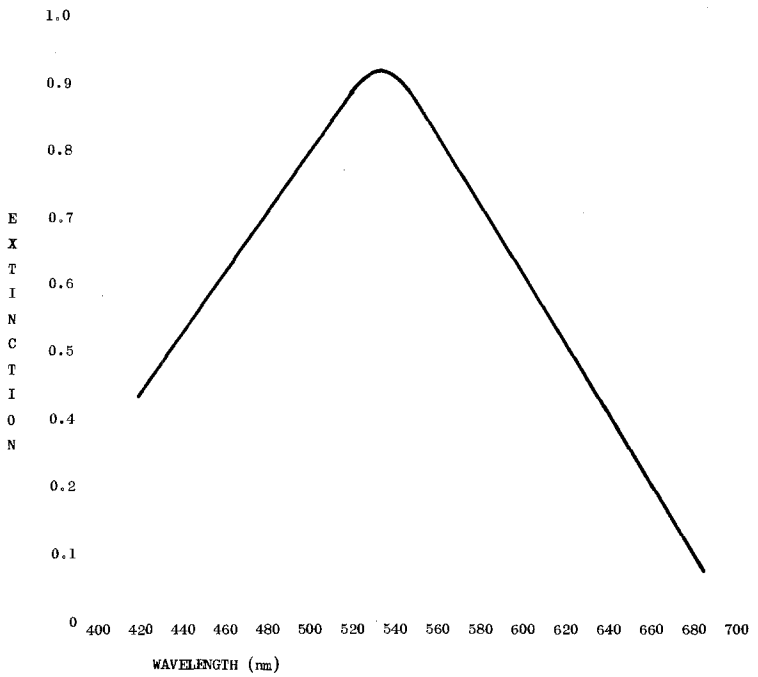

Fig. 1. Average extinction plotted against wavelength for a P.A.S. stained section cut at 8 μ

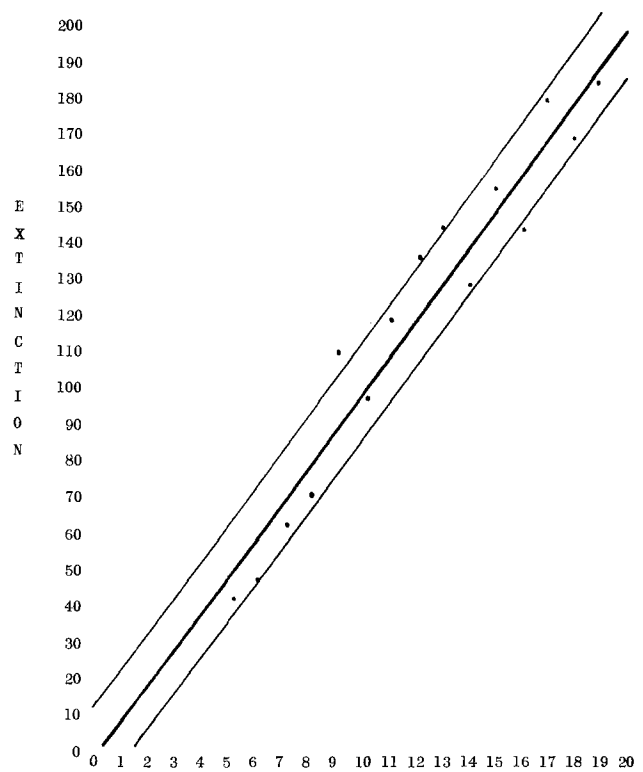

Fig. 2. Average extinctions plotted against corrected section thickness of P.A.S. stained sections of the corpus callosum

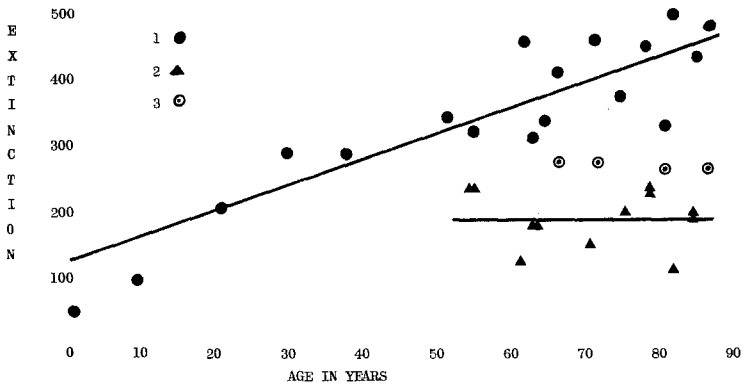

Fig. 3. The average extinction plotted against age for 30 neurones in each layer 5 and 6 for 1. Controls 2. Blind and 3. Apparently normal but demented or deaf cases in 16 μ P.A.S. stained sections

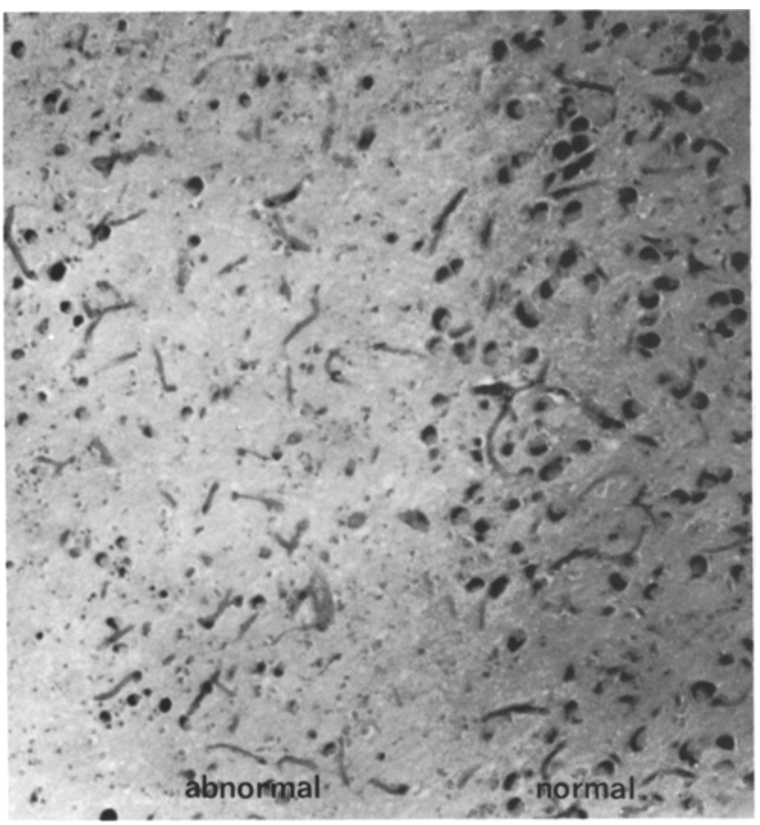

Fig. 4. A photomicrograph of a 16 μ P.A.S. stained section showing normal layer 5 and visually deprived layer 6 (×140)

Discussion

A linear relationship between section thickness and density of the colour produced has been demonstrated in this study and shows that it can be assumed that the reaction product in the sections obeys Beer's law. It is postulated that the dye complex is formed by a polyaldehyde, most likely to be a glycoprotein as Leblond et al. (1957) suggest, having found that the intensity of P.A.S. staining in tissue sections corresponds closely with the quantity of hexose sugars present. These authors also say that glycoproteins are the only sustances likely to react in paraffin sections.

1, 2−glycol linkages have been described in ceroid (Lillie, 1952), and early stage in the development of lipofuscin, and hexose sugars are present in the sphingomyelin component of lipofuscin (Björkerud, 1964). While the Schiff reaction must be regarded as a reagent for aldehyde groups (Pearse, 1972) the source of all the aldehyde groups produced by oxidation of lipofuscin with periodic acid is obscure.

The results of the second part of our study demonstrate that the P.A.S. positive component of lipofuscin increased linearly with age as does that component which fluoresces with UV light (Mann and Yates, 1974). This suggests that the pool of P.A.S. positive material increases with continued cell function but does attain a stable end value since the lipid is oxidised further in the development of more mature lipofuscin. The reduced quantity of the P.A.S. positive component of lipofuscin in the cases with blindness following transsynaptic degeneration, lipofuscin, the waste product of oxidation of lipids, does not accumulate or mature. Oxidative enzymes have been shown to be reduced in neurones of the L.G.B. in experimental animals with lesions in the optic pathways (Cotlier et al., 1965), and we suggest that the P.A.S. reaction may be used to monitor transsynaptic degeneration by examining the products of oxidation.

In those cases where the amount of lipofuscin was found to be reduced in association with dementia it is postulated that there is reduced L.G.B. neuronal fuction although microscopic examination of the L.G.B. using standard neruopathological techniques failed to show any abnormality. These findings suggest that elderly patients with organic dementia may have impaired visual function which has not been identified clinically because of the difficulty of the careful visual assessment of such individuals.

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