Letters to the Editor

⁶ De Bruyn AM, Klein F, Neumann H, Sankuyl LA, Vermeern R, Le Blansch G. The absolute quantification of human IgG: standardisation and normal values. *J Immunol Methods* 1982;48:339-48.

Possible role of deficiency of selenium and vitamin E in atherosclerosis

Ellis *et al*¹ draw attention to the enigma of the association of selenium and vitamin E deficiency with coronary artery disease. One theoretical explanation is provided by the hypothesis that monocyte-derived macrophages in the early plaque are causing damage by oxidising the lipids they contain.^{2,3} This springs from the observation that macrophage like cells in even the earliest human plaques contain ceroid, the production of which might well be preceded by release of diffusible oxidised lipids that are cytotoxic.^{3,4} Further, the insoluble ceroid appears to be laid down around the periphery of soluble lipid droplets in membrane bound vesicles and persists in the necrotic base of advanced plaques.⁴⁻⁶ The central, soluble lipid may be difficult to disperse in vivo because of this skin of ceroid. This activity of the macrophage's microbicidal oxidative systems might prove an important, or even essential, stage in the development of the plaque. Deficiency of antioxidants or components of antioxidants would therefore accelerate the disease.

Further work to test this hypothesis is in progress in this laboratory.

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⁵ Mitchinson MJ, Ball RY, Brooks PN. Ceroid in atherosclerosis. *J Pathol* 1984 (in press).

⁶ Mitchinson MJ, Ball RY, Hothersall DC, Brooks PN, de Burbure CY. The distribution of insoluble lipid in human aortic atherosclerosis. (Submitted for publication).

Localisation of aluminium and iron by histochemical and laser microprobe mass analytical techniques in bone marrow cells of chronic haemodialysis patients

We wish to corroborate the observations of Dr Kaye,¹ who noticed a positive aluminon staining² in the bone marrow cells of some patients with end stage renal disease. The positive staining in the bone marrow cells is putatively regarded as caused by aluminium storage in unidentified cells, possibly of the macrophage system. Iron stain, however, was negative. This is surprising, since by Prussian blue staining for iron heavy iron loading is often found in bone marrow macrophages of chronic haemodialysis patients.³

We studied transiliac bone biopsies (obtained with a 7 mm diameter trephine⁴) of three chronic haemodialysis patients (A, B, and C), who showed aluminium induced osteomalacia.⁵ Patients A and B showed considerable bone marrow iron storage, in contrast to patient C. Bone aluminium and iron concentrations (microgram of the element per gram of bone, wet weight) were determined using electrothermal atomic absorption spectrometry, as described elsewhere.⁶ Respective bone aluminium concentrations were 25.2 µg/g, 63.1 µg/g, and 83.6 µg/g, and the corresponding iron concentrations were 736 µg/g, 386 µg/g, and 64 µg/g. For histological examination the bone specimens were fixed in Burkhardt's solution for 24 h⁷ and subsequently transferred to absolute methanol. The specimens were embedded in methyl methacrylate and sections were cut from the undecalcified biopsies with a Jung K sledge microtome. Eight micrometer thick sections were stained by means of Goldner's method⁸ for qualitative histology, and 2 µm sections were aluminon stained.²

Patient A showed very few bone marrow cells with positive aluminon staining, while in the bone marrow of patient C numerous bright red cells were seen. The number of aluminon stained cells in patient B was intermediate between those of patients A and C. Histologically, these cells appeared to be macrophage in type, as were those reported by Dr Kaye.¹

In order to verify the results of the aluminon staining, laser microprobe mass

analysis (LAMMA)⁹ was performed on the aluminon stained sections. With LAMMA, which provides multi-element mass spectra at the ultrastructural level,⁹ not only iron but also aluminium was detected in many of the histochemically aluminon negative bone marrow cells of patients A and B (Figure). On the other hand the mass spectra of the aluminon positive bone marrow cells of patient C showed aluminium and no appreciable iron signal. According to the LAMMA spectra, the amounts of aluminium in the histochemically aluminon negative cells of patients A and B were of the same order of magnitude as those in the aluminon positive cells of patient C.

Our findings are in conflict with the supposition of Dr Kaye¹ that aluminon staining is unable to demonstrate aluminium in bone marrow cells in methyl methacrylate embedded sections. That aluminon positive marrow cells had not been previously reported might have been due to the different fixation procedures used by other authors.

The aluminon staining of undecalcified bone sections shows the presence of aluminium with high specificity. Positive histochemical staining with aluminon at the osteoid/calcified bone boundary has been confirmed with several microanalytical methods.⁹⁻¹¹ Nevertheless, LAMMA appears to demonstrate aluminium in bone marrow cells, which contain much iron and are histochemically aluminon negative. The LAMMA results presented here indicate that aluminium and iron may be stored concomitantly in bone marrow cells of chronic haemodialysis patients. This observation provides additional support to the assumption of Dr Kaye that the aluminium storing bone marrow cells are indeed macrophages. The concurrent storage of iron and aluminium in bone marrow macrophages is a striking analogy to the accumulation of both elements in hepatocyte and Kupffer cell lysosomes of patients with iron and aluminium overload⁸ and to their concomitant occurrence at the osteoid/calcified bone boundary.³

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