Letters to the Editor

Reassignment of gram/litre values for international immunoglobulin standards

In 1980¹ it was recommended that the old WHO Standard 67/69 should be abandoned. At that time there was no alternative but to transfer its assigned gram/litre values for IgG, IgA, and IgM to our new standard IFCC 74/1. The latter standard is now eight years old, and readers might be interested to know that there is now preparation number three: the first British standard for human serum protein (74/520), which is available from the National Institute for Biological Standards and Control, Hollyhill, Hampstead, London NW3 6RB. Standard 74/520 has undergone calibration against three reference preparations for IgA, IgM, and IgG² and with other American standards,³ with new assigned mass values about 7% higher than those originally given to WHO 67/69.

Such standards, together with IFCC 74/1, have been used to calibrate a new standard serum available from Dr A Milford Ward, Supraregional Protein Reference Unit, Department of Immunology, Royal Hallamshire Hospital, Sheffield S10 2RX, which is to be called SPS-01. The new assigned values are given in Table 1.

These new values reset the existing normal ranges for Caucasians in this country. Table 2 has been produced in a form whereby it can replace page 134 of the SAS booklet. In conforming to international opinion, the IgG value of IFCC 74/1 was reduced to 8.67 g/l,⁴ but it is now restored to 9.34 g/l. In 1966, and again in 1971,⁵ mean normal adult serum was assigned a value of 9.47 g/l, so it has taken 16 years to return to within 1% of that original assignment. IgA is still assigned lower than my own estimate⁵ (which agreed with that of Joseph Heremans) and the IgM value is a little high compared with what others in Europe^{5,6} believe to be the true value. Nevertheless, for the sake of international comparison the directors of the Supraregional Protein Reference Units of Great Britain recommend that we all use the values given in Table 1. It is up to the individual whether he wishes to use the normal ranges given in Table 2.

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- ⁴ Hobbs JR, Harboe N, Alper C, Johansson BG. The human serum standard IFCC 74/1. *Chim Acta* 1971; **98**:179F-86F.
- ⁵ Hobbs JR. Immunoglobulins in clinical chemistry. *Adv Clin Chem* 1971; **14**:219-317.

Table 1 Three reference standard preparations available in the United Kingdom with their newly assigned gram equivalents.

Standard	Immunoglobulin concentration (g/l)		
	IgG	IgA	IgM
IFCC 74/1	9.34	1.67	1.26
SPS 01	10.41	1.97	1.29
74/520*	7.36	1.43	0.83

*When reconstituted by the addition of 1 ml of water.

Table 2 Normal ranges for serum immunoglobulin concentrations in Caucasians (-2 SD Median, + 2 SD by Log-Gaussian) as determined in 53 males and 54 females above 15 years; cohorts of at least 30 subjects for the others. These are adjusted to the new g/l equivalents for International Standards (November 1982)

Age	Immunoglobulin concentration (g/l)		
	IgG	IgA	IgM
Cord	10.8 5.2-18.0	— <0.02	0.1 0.02-0.4
Weeks			
0-2	9.4 5.0-17.0	0.02 0.01-0.08	0.1 0.05-0.4
2-6	7.1 3.9-13.0	0.05 0.02-0.15	0.2 0.08-0.4
6-12	3.9 2.1-7.7	0.15 0.05-0.4	0.4 0.15-0.7
Months			
3-6	4.6 2.4-8.8	0.2 0.1-0.6	0.6 0.2-1.0
6-9	5.2 3.0-9.0	0.3 0.15-0.7	0.8 0.4-1.6
9-12	5.8 3.0-10.9	0.4 0.2-0.7	1.2 0.6-2.1
Years			
1-2	6.4 3.1-13.8	0.7 0.3-1.2	1.3 0.5-2.2
2-3	7.0 3.7-15.8	0.8 0.3-1.3	1.3 0.5-2.2
3-6	9.9 4.9-16.1	1.0 0.4-2.0	1.3 0.5-2.0
6-9	9.9 5.4-16.1	1.3 0.5-2.4	1.2 0.5-1.8
9-12	9.9 5.4-16.1	1.4 0.7-2.5	1.1 0.5-1.8
12-15	9.9 5.4-16.1	1.7 0.8-2.8	1.2 0.5-1.9
15-45	9.9 5.4-16.1	1.9 0.9-3.4	1.2 0.5-2.0
>45	9.9 5.3-16.5	1.9 0.8-4.0	1.2 0.5-2.0

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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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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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