



**Fig. 1** Sucrose density gradient analysis of *a*, garter snake serum; *b*, bile; and *c*, succus entericus (SE). Bile was aspirated from the gall bladders of snakes and then pooled, dialysed and concentrated by negative pressure dialysis. SE was obtained from the intestines as described before<sup>5</sup>. 10–44% linear sucrose gradients were centrifuged for 18 h at 35,000 r.p.m. as described previously<sup>5</sup> using <sup>125</sup>I-labelled rabbit gamma globulin (— · —) and <sup>131</sup>I-labelled hog thyroglobulin (—) as 7S and 19S markers, respectively. The fraction number is represented on the abscissa, relative IgM concentration and c.p.m. on the ordinate. Fractions (0.1ml) were removed from the top of the gradients and analysed for IgM concentration by the Laurell antibody-Agarose technique<sup>6</sup> using specific rabbit anti-garter snake IgM, produced by injection of 200  $\mu$ l of concentrated bile (equivalent to neat bile from two snakes) in an emulsion prepared with equal volumes of M/15 PBS and Freund's complete adjuvant (Difco). Because emulsification of bile was very difficult it was necessary to prepare the emulsion in a Sorvall Omni-Mixer using 10 ml of PBS and 10 ml of adjuvant. Rabbits were injected subcutaneously at multiple sites and were boosted 3 and 6 weeks later with 100  $\mu$ l of bile in incomplete Freund's adjuvant (Difco). After absorption (see text) the antiserum reacted only with IgM in serum or secretory fluids. The approximate S values of the IgM concentration peaks were: *a*, serum  $\approx$  20.5S; *b*, bile  $\approx$  17S; and *c*, SE  $\approx$  15S and 20.5S. . . . ., IgM.

in turtles (*Chrysemys picta*) and bullfrogs (*Rana catesbeiana*) because two rabbits immunised with neat bullfrog bile and two rabbits immunised with turtle bile each produced antisera which reacted specifically with their respective serum IgM. None of the antisera detected antigenic differences between serum and secretory IgM. It was particularly interesting that rabbit anti-snake, bullfrog and turtle bile revealed no cross reaction between the serum IgM of these species. In contrast, bullfrog anti-rabbit gamma globulin showed broad cross reactivity with other mammalian gamma globulins<sup>8</sup>. This phenomenon is discussed further in ref. 6.

It is noteworthy that the production of antisera specific (except for anti- $\alpha$  globulins) for snake, turtle or bullfrog IgM was achieved simply by injection of rabbits with bile from the respective species. Purified snake serum IgM, however, elicited abundant cross reacting (L chain) antibody. We are investigating whether these specificity differences resulted from unique properties of secretory IgM or from differences between the immunising emulsions (see legend to Fig. 1).

Sequence analysis of human Ig has revealed marked structural similarities between IgM and IgA<sup>2,7</sup>. These two Ig classes also resemble one another functionally, in that IgM can substitute for IgA in external secretions of patients with selective IgA deficiency<sup>8</sup>. The results of our study indicate that IgM served the function of a secretory Ig before the evolution of the  $\alpha$ -heavy (H) chain gene. If sequence analysis of avian secretory Ig corroborates its homology with mammalian IgA, the lack of a unique class of secretory Ig in present day reptiles would place the evolution of the  $\alpha$ -H chain gene in a high reptilian ancestor of birds and mammals.

J. L. PORTIS  
J. E. COE

National Institute of Allergy and Infectious Diseases,  
National Institutes of Health,  
Rocky Mountain Laboratory, Hamilton, Montana 59840

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## Corrigendum

The complete authorship of the article "Age of KBS Tuff in Koobi Fora Formation, East Rudolph, Kenya" (*Nature*, **258**, 395; 1975) should be:

G. H. Curtis, R. E. Drake, T. E. Cerling, B. L. Cerling and J. H. Hampel.

## Erratum

In the article "Post-transcription control of isocitrate lyase induction in the eukaryotic alga *Chlorella fusca*" by A. H. Scragg, P. C. L. John and C. F. Thurston (*Nature*, **257**, 498; 1975) the second line of text below the legend to Fig. 2 should read . . . concentration was largely isocitrate lyase. This, in turn, demonstrated . . . and not as printed.