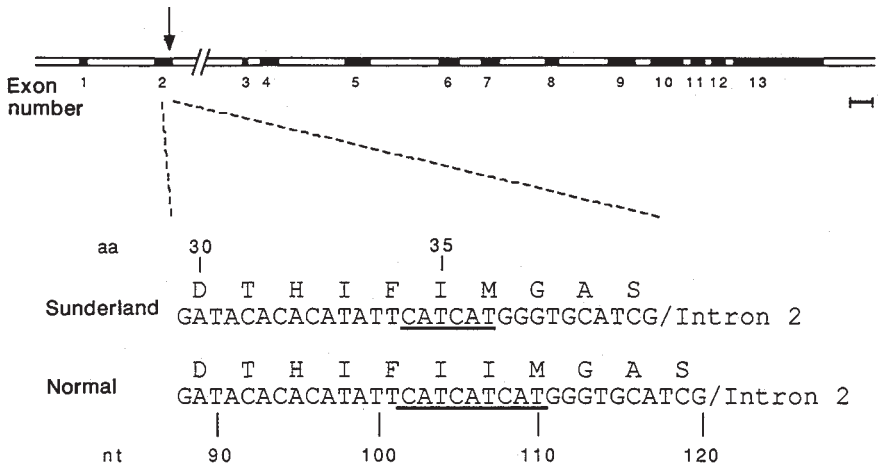
Deficiency in red blood cells

SIR — Glucose 6-phosphate dehydrogenase (G6PD) is a ubiquitous enzyme encoded by a gene that is highly polymorphic in humans[1]. Its deficiency is common in populations that have been subjected to selection by *Plasmodium falciparum* malaria[2]. Common variants entail the risk of acute haemolytic anaemia, but clinical expression of these variants is generally mild. By contrast, some individuals have rare variants associated with much more severe haemolysis[3]. The gene encoding G6PD has been mapped on the X-chromosome in mammals, specifically on Xq28 in humans[4].

Kanno *et al.* claimed[5] that G6PD in red cells consisted of an amino-terminal region encoded by a gene on chromosome 6, and of a carboxy-terminal region encoded by the X-linked gene. In a News and Views article written at the time[6], L.L. commented that, although several arguments could be made against the likelihood of such a fusion protein, formal genetic evidence for X-linkage of the amino-terminal region of G6PD was not available, because none of the mutations reported at the time[7] mapped to that region. The claim by Kanno *et al.* has since been refuted by molecular analysis[8,9] and attributed to a purification artefact[10]. Nevertheless, mutations in the amino-terminal region have still failed to turn up[11].

Using a polymerase-chain-reaction based technique[12], we have now determined the nucleotide sequence of the entire coding region of the G6PD gene from a person with severe red cell G6PD deficiency and chronic haemolytic anaemia. The only abnormality we found was a three base-pair deletion in exon II (see figure), which predicts the loss of one of two adjacent isoleucine residues (amino acid 35 or 36), just upstream of the methionine residue called 'junctional' by Kanno *et al.* This part of exon II would not be expressed in red cells if the claim of Kanno *et al.* had been correct. Our observation demonstrates that a mutation in this X-linked amino-terminal region of G6PD causes deficiency in red cells.

This new type of G6PD, which we designate G6PD Sunderland, is the first variant found to result from a deletion rather than a base change. The deletion is within a threefold CAT repeat, and has presumably arisen through misalignment at meiosis, with conservation of the reading frame.



Genomic structure of the human G6PD gene (top). The break symbolizes the large intron II. The arrow indicates the position of the mutation in G6PD Sunderland. Scale bar, 200 base pairs. Below, Amino acid and nucleotide sequence of a portion of exon II of G6PD Sunderland and of normal human G6PD. Amino acid (aa) numbers are on top, and nucleotide (nt) numbers are at the bottom. Numbering is from the beginning of the translated sequence (see in ref. 7). The CAT repeats, one of which is deleted in G6PD Sunderland, are underlined.

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1. Luzzatto, L. & Battistuzzi, G. *Adv. hum. Genet.* **14**, 217–329 (1985).
2. Luzzatto, L. *Blood* **54**, 961–976 (1979).
3. Luzzatto, L. & Mehta, A. in *The Metabolic Basis of Inherited Disease* (eds Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D.) 2237–2265 (McGraw-Hill, New York, 1989).
4. Pai, G. S., Sprenkle, J. A., Do, T. T., Mareni, C. E. & Migeon, B. R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2810–2813 (1980).
5. Kanno, H., Huang, I.-Y., Kan, Y. W. & Yoshida, A. *Cell* **58**, 595–606 (1989).
6. Luzzatto, L. *Nature* **341**, 286–287 (1989).
7. Vulliamy, T. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 5171–5175 (1988).
8. Mason, P. J., Bautista, J., Vulliamy, T. J., Turner, N. & Luzzatto, L. *Cell* **62**, 9–10 (1990).
9. Beutler, E., Gelbart, T. & Kuhl, W. *Cell* **62**, 7–8 (1990).
10. Yoshida, A. & Kan, Y. W. *Cell* **62**, 11–12 (1990).
11. Beutler, E. *Semin. Hemat.* **27**, 137–164 (1990).
12. Poggi, V., Town, M., Foulkes, N. S. & Luzzatto, L. *Biochem. J.* **271**, 157–160 (1990).

Sea-level constraints

SIR — The authors of three important papers[1–3] used Barbados coral cores of latest Pleistocene and Holocene ages to provide important constraints on sea-level fluctuations and to improve the radiocarbon timescale in this interval by calibrating it against the uranium–thorium timescale. The results of Bard *et al.*[3], for example, indicate that the last deglaciation started about 3,000 years earlier when referenced to the U–Th timescale than when referenced to the radiocarbon timescale. These authors suggest that this difference may explain the previously noted discrepancies between observations of sea-level change and models of glacial rebound in which ice sheets are considered only over Laurentia and Fennoscandia and that it is not necessary to introduce meltwater into the oceans from other ice sheets[4–6].

This conclusion is incorrect. Time enters into the formulation in three ways: through the history of the changes in the ice sheets; through the observations of sea-level change and through the Earth's rheology. Provided the timescales are linear relative to each other and provided that the same timescale is used throughout, the use of either the U–Th or the ^{14}C timescale is immaterial. The results of Fairbanks[1] indicate that the relation between the ^{14}C and U–Th timescales is effectively linear for the past 20,000 years and that any departures from this linearity are small when compared with the uncertainties associated with many of the radiocarbon ages used to constrain ice models and sea-level change.

The ice models are almost wholly constrained by radiocarbon ages of debris left by the retreating ice front. In some instances in Fennoscandia, varve chronologies have been used; these should be reduced to the radiocarbon timescale. (This does not appear to have been done but there are only few varve dates so they are unlikely to distort the global ice-sheet models.) Observations of sea level have sometimes been related to varve or U–Th timescales, but the bulk of reported data refers to the conventional radiocarbon timescale. In the rebound modelling, the Earth's viscous time constraint(s) are usually considered as unknowns so that the viscosities will be either in Pa (^{14}C)s or Pa (U–Th)s, depending on which timescale is used for the other two inputs. (The distinction is not so insignificant if the two timescales differ by about 3,000 years after 20,000 years.)

If the U–Th timescale for the Barbados corals is used to constrain the glacial-hydroisostatic rebound models then the ice models must be transformed to this timescale as well, with the result that the relation between observation and model prediction will be unchanged unless the relation between the two timescales is significantly nonlinear. One cannot usefully compare the U–Th

timescale-controlled sea-level data with rebound predictions based on ice models linked to the ^{14}C scale. Yet this is what Bard *et al.* do.

A second difficulty with the conclusion of Bard *et al.* is the sea level during the last glacial maximum. Around 18,000–20,000 years ago, sea levels are generally believed to have been at about 130 m below their present level (although this depth will be regionally variable) and the Barbados coral data support this: the minimum depths at this time of the corals are about 120 m below present sea level. Ice models for the Laurentide and Fennoscandia that produce rebound at the centres of these ice sheets that are consistent with these observations of sea-level change within and near the ice-sheet margins contain only enough ice to raise sea level by 60–80 m (refs 5,7). Increasing the volumes in these ice sheets results only in implausible predictions of rebound at these sites unless the melting was initially very rapid. The Barbados sea-level curve from 18,000 to 6,000 years before present, however, argues against this, so it has been suggested that this missing melt water has to come from other areas such as the Barents–Kara ice sheet in Antarctica, or possibly from eastern Siberia[4–6]. Rather than negate the previously drawn conclusions about the importance of these centres of deglaciation, the Barbados results reinforce them.

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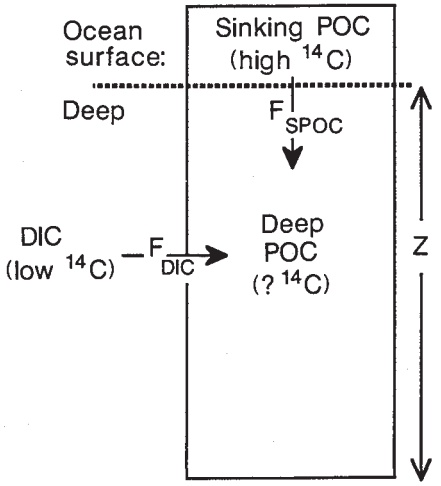
1. Fairbanks, R.G. *Nature* **342**, 637–642 (1989).
2. Bard, E., Hamelin, B., Fairbanks, R. G. & Zinder, A. *Nature* **345**, 405–410 (1990).
3. Bard, E., Hamelin, B. & Fairbanks, R. G. *Nature* **346**, 456–458 (1990).
4. Nakiboglu, S. M., Lambeck, K. & Aharon, P. *Tectonophysics* **91**, 335–358 (1983).
5. Nakada, M. & Lambeck, K. *Nature* **333**, 36–40 (1988).
6. Peltier, W. R. *Quat. Res.* **29**, 93–112 (1988).
7. Peltier, W. R. & Andrews, J. T. *Geophys. J. R. astr. Soc.* **46**, 605–646 (1976).

Another recipe for bomb ^{14}C dilution

SIR — As recently pointed out by Druffel and Williams[1] and Toggweiler[2], the relatively low amount of atom-bomb-derived ^{14}C in deep-ocean particulate organic carbon (POC) has significant implications for the way carbon is cycled in the ocean's interior. Druffel and Williams conclude that the low $\Delta^{14}C$ found in POC suspended in the deep ocean is probably the result of dilution by incorporation of ^{14}C-depleted dissolved organic carbon (DOC) into deep POC. As well as DOC→POC transformations, I wish to point out that dissolved inorganic carbon (DIC) is another large carbon source for such dilution, and that the conversion rate of DIC to POC in the deep sea is quantitatively, and therefore isotopically, important.



Model of deep-ocean POC formation from dissolved inorganic carbon (DIC) and sinking POC sources. Within the deep ocean, sinking and suspended POC are not differentiated. F_{spoc1}, areal flux rate of sinking POC through the surface/deep ocean boundary; F_{dic}, areal flux rate (uptake) of deep DIC into POC.

Let us assume that the only source of non-bomb carbon in the deep ocean is DIC, and that deep POC must therefore originate from some combination of this source and ^{14}C-rich sinking POC (see figure). If we know the fluxes and $\Delta^{14}C$ of these sources, the $\Delta^{14}C$ in POC resident in the deep ocean can be calculated. Although it has been assumed[1] that uptake of DIC into POC is negligible in the deep sea, Sorokin reported[3] uptake rates ranging from 0.5 to 3 μg C m^{-3} d^{-1} at depths greater than 1 km in the central Pacific Ocean. Such rates are indeed minuscule when compared to those in surface waters, but they become significant when integrated over depth and compared with the sinking POC flux into the deep ocean. Using a mean deep-ocean DIC uptake rate of 2 μg C m^{-3} d^{-1}, the estimated areal flux rate of DIC into POC (10 mg C m^{-2} d^{-1}) equals the sinking POC flux into deep water in the north central Pacific Ocean (see table). This yields an estimated deep POC $\Delta^{14}C$ of −52 ‰ (see table), even more depleted in bomb ^{14}C than actually observed[1] (> 43 ‰), indicating the potential importance of the DIC → POC pathway in effecting ^{14}C dilution in the deep sea.

In the Santa Monica Basin, a greater flux (30 mg C m^{-2} d^{-1}) of POC sinking into a shallower aphotic zone could be viewed as less advantageous for dilution of bomb ^{14}C in deep POC. But aphotic DIC→POC conversion in such basin environments ranges from <1 to 24 mg C m^{-3} d^{-1} (refs 4–7), at least several orders of magnitude greater than those observed[3] in the open ocean. Assuming a conservative mean rate estimate for this site of 1 mg C m^{-3} d^{-1}, depth-integrated uptake of deep DIC is calculated to be 800 mg C m^{-2} d^{-1}, or more than 20 times the flux entering aphotic waters via sinking POC. This again yields an estimated deep POC $\Delta^{14}C$ value that is much lower than that of POC sinking from surface waters (see table).

A further example of the magnitude of such a process is provided by Tuttle and Jannasch[5], who reported that DIC uptake in waters above the Cariaco trench is at least 17–58 % as great as photosynthetic inorganic carbon assimilation in overlying surface waters. The above observations again lead to the conclusion that bomb ^{14}C in resident deep-ocean POC can be substantially diluted by DIC uptake, independent of DOC incorporation or other mechanisms[1].

FLUXES AND $\Delta^{14}C$ RELEVANT TO DEEP POC FORMATION

Site	Z	Fluxes SPOC	Fluxes DIC	$\Delta^{14}C$ SPOC	$\Delta^{14}C$ DIC	$\Delta^{14}C$ DPOC
	(m)	(mg C m^{-2} d^{-1})		(‰)		
NCP	4,760	10	10	+136	−240	−52
SMB	800	30	800	+86	−50	−45
Ref.	1	8,9	3–7	1	1	(This report)

SPOC, sinking POC at surface/deep ocean boundary; DPOC, deep POC (both sinking and suspended); NCP, north central Pacific Ocean site[1]; SMB, Santa Monica Basin site[1]. The DIC flux (F_{dic}) is derived by multiplying the mean per-volume rate estimates (2 μg C m^{-3} d^{-1} (ref. 3) and 1 mg C m^{-3} d^{-1} (refs 4–7) for NCP and SMB, respectively) times the respective deep-ocean water column depth, *Z*. The surface/deep ocean boundary is arbitrarily chosen to be at a depth of 1,000 m at the NCP site and at 100 m at the SMB site. DPOC $\Delta^{14}C = ((F_{spoc}/F_t) \times SPOC\ \Delta^{14}C) + ((F_{dic}/F_t) \times DIC\ \Delta^{14}C)$ where $F_t = F_{spoc} + F_{dic}$. Δ^{14} (ref. 1) in units of parts per thousand (‰).

Thus, as previously noted[10], DIC uptake should not be ignored in interpreting the paucity of ^{14}C in some deep-sea organisms and POC pools. It would therefore be premature to ascribe such ^{14}C depletion to DOC incorporation alone. Clearly, further rate and isotope measurements are needed better to characterize this important piece of the global carbon cycle.

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1. Druffel, E.R.M.. & Williams, P.M. *Nature* **347**, 172 (1990).
2. Toggweiler, J. R. *Nature* **347**, 122 (1990).
3. Sorokin, Yu. I. *Oceanology* **11**, 85 (1971).
4. Seki, H. & Zobell, C. E. *J. Oceanogr. Soc., Japan* **23**, 182 (1967).
5. Tuttle, J. H. & Jannasch, H. W. *Limnol, Oceanogr.* **24**, 746 (1979).
6. Seki, H. *et al. Arch. Hydrobiol.* **100**, 73 (1984).
7. Juniper, S. K. & Brinkhurst, R. O. *Mar. Ecol. Prog. Ser.* **33**, 41 (1986).
8. Martin, J. H. *et al. Deep-Sea Res.* **34**, 267 (1987).
9. Nelson, J. R. *et al. Cont. Shelf Res.* **7**, 307 (1987).
10. Rau, G. H., Karl, D. M. & Carney, R. S. *Deep-Sea Res.* **33**, 349 (1986).

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