complexes with histidine and methionine as axial haem ligands (as in cytochromes *c*) values for uncorrected E'_0 of about −0.10 V have been obtained[3,4], with one histidine (as in haemoglobins) at about −0.16 V and with two histidines (analogous to cytochromes *b*) at −0.21 V. Thus, for different haem proteins, different corrected E'_0 values are obtained when the same value of increment $\Delta E'_0$ is added.

Moreover, from the viewpoint of haem accessibility, the lower $\Delta E'_0$ corresponds, according to Kassner[2], to the better accessibility of haem. Stellwagen[1] presented the data of haem accessibility (cytochromes *c* about 5% haem exposed, haemoglobin subunits ~16–18%, cytochrome b_5 ~23%). Thus, the highest values of E'_0 must be obtained for the cytochromes *c* because of both high E'_0 of model system and high $\Delta E'_0$; and lowest for the cytochrome b_5 (the lowest values of E'_0 and $\Delta E'_0$). This is, however, in good correlation with the experimental values of E'_0 as presented by Stellwagen[1] (average 0.280 V for cytochromes *c*, 0.080 V for haemoglobin subunits and 0.020 mV for cytochrome b_5).

Thus, it is most probable that both the effect of haem axial ligands and the nature of haem surroundings determine the actual value of oxidation–reduction potential. This may be crucial in discussing, for example, the high negative values of E'_0 of cytochromes P-450 (ref. 5), where one of the axial ligands is presumably the mercaptide (anionic) sulphur[6].

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STELLWAGEN REPLIES—Anzenbacher correctly points out that the Kassner proposal concerns the $\Delta E'_0$ resulting from complexation of haem by apoprotein without changing the nature of the axial ligands and suggests that the E'_0 values for the haem octapeptide from cytochrome *c* having histidine in the fifth coordination position and either water, imidazole acetylmethionine in the sixth coordination position would serve as appropriate model haems for the haemoproteins considered. However, for purposes of surface area calculation it should be recognised that the atoms of the octapeptide very likely shield part of the haem from the solvent owing to the three point attachment of haem to octapeptide (two thioether bridges and one coordinate : covalent bond). While the surface area of a haem coordinated with either one or two histidines or one histidine and one methionine could be calculated by elimination of the remaining protein atoms in the crystallographic models of appropriate haem proteins, the E'_0 values of these coordinated haems would not necessarily be identical with E'_0 of the octapeptide models owing to potential differences in the geometry of the axial coordinate : covalent bonds. However, assuming that the E'_0 values of the octapeptide models are appropriate and that the haem exposure in each model is the same, recalculation of Fig. 2 of my paper $\Delta E'_0$ values yields essentially the same relationships. In any case, the major point remains, namely that the apolarity of the haem crevice or of total haem environment seems independent of E'_0 or $\Delta E'_0$ for haemoproteins having either one or both axial ligand identical.

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Effect of chloroquine on Epstein–Barr virus expression

THE finding by Karmali *et al.*[1] that 'chloroquine enhances Epstein–Barr virus expression' was of great concern to us as it implies that chloroquine, through its effect on the virus, might stimulate the development of Burkitt's lymphoma (BL). We are using chloroquine to suppress malaria in a child population in Tanzania in an attempt to reduce the incidence of BL. A BL-promoting effect of chloroquine might tend to mask any reduction in BL incidence brought about by malaria suppression and would greatly complicate the interpretation of the findings in our trial.

We have therefore repeated and extended Karmali's experiments in our laboratory. This type of experiment is frequently carried out by us, as studies on the expression of Epstein–Barr virus (EBV) antigens is one of our major topics of interest[2]. We found that, as Karmali reported, chloroquine had no effect on spontaneous expression of EBV antigens in either non-producer (Raji) or producer (P3HR-1 or B95) lines. We also found no effect of this compound on chemical induction of early antigen (EA) in Raji cells. Furthermore, in an attempt to repeat Karmali's experiment using superinfected Raji cells, we were unable to reproduce his results.

In our hands, whatever the multiplicity of infection, dose or origin of chloroquine used (from different commercial firms, including the one used in Karmali's experiment), we found no effect of chloroquine on expression of EBV antigens. We therefore feel that Karmali's conclusions should be reconsidered, for even if it were true that expression of EBV antigens could be enhanced by chloroquine in the system he describes, this system is very particular, as the P3HR-1 EB virus is a unique laboratory 'strain' which has lost one of the major properties of all other EBV isolates, its transforming ability. Moreover, in all other systems of EBV antigen synthesis studied, chloroquine has no effect.

In the absence of significant effects of chloroquine on EBV expression in the laboratory and as no effect of chloroquine medication on EBV serology has ever been reported in the many populations who receive chloroquine for malaria prophylaxis, we are convinced that Karmali's conclusions regarding the effect of chloroquine on BL development are premature. We are, therefore, continuing our studies of malaria suppression and BL incidence, as originally planned.

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KARMALI AND HORROBIN REPLY—We have reviewed our work carefully. The observations were made blind by three separate individuals and we stand by our findings. Minor differences of technique can lead to different results and we have no explanation for the discrepancy. We agree with Lenoir and Geser that the chloroquine trial should not be stopped because of a laboratory observation, for such observations in highly artificial conditions may be poor guides to *in vivo* results. Nevertheless, our work does sound a note of caution and we would argue that the trial should be monitored exceptionally carefully and any evidence of increased rather than reduced Burkitt lymphoma incidence investigated immediately.

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