virtually indistinguishable. However, Hb SS in aerobic conditions shows marked enhancement of invasion and development rather than, as suggested by the authors, suppression of growth in conditions of low oxygen tension. One accepts, of course, the statistical limitations of the relatively few experimental results for Hb SS.

Pasvol *et al.* also maintain that their hypothesis is supported by the data in their Tables 3 and 4. This interpretation ignores the significance of the large number of abnormal forms observed for red cells maintained in conditions of low oxygen tension (Table 3—74% for Hb AS; 20% for Hb SS—and Table 4). Furthermore, the use of a growth score from 1 to 6 for the different stages of parasite development, with abnormal forms being given an arbitrary value of 0, seems unjustifiable in the likely event that these abnormal forms represent the growth of the parasite to a stage at which no further development was possible, thus aborting the parasite life cycle and producing degenerate forms.

Table 1 Mean and s.e. for the number of ring-forms per 100 red cells after 12 h in aerobic or low oxygen conditions (data from Pasvol *et al.*[1])

	Aerobic	Low oxygen	
Haemoglobin			
AA	11.71±1.48	12.32±1.23	(13)
AS	11.52±1.31	10.35±1.34	(11)
SS	18.13±2.48	11.73±1.01	(4)

We have recently suggested that production of fatty acids by *Plasmodium knowlesi* and *Plasmodium berghei* may represent the molecular mechanism by which the plasmodial parasite damages red cells and produces haemolysis which may often be out of all proportion to the parasitaemia[2,3]. Similar evidence for the involvement of fatty acids in *Plasmodium lophurae* infections has been reported[4], and the whole question has been reviewed by Holz[5]. The fatty acids within the red cell are buffered by the haemoglobin that is present at high concentration, and Hbs AS, SS and SC are more effective than Hb AA at buffering fatty acids. This increase in buffering capacity has been rationalised at the molecular level in terms of the change in the amino acid residue at the 6β position[3,6], and could represent one of the mechanisms by which Hb protects against *P. falciparum* malaria.

In conclusion, we suggest that the data presented by Pasvol *et al.*[1] do not support their hypothesis concerning the mechanism for protection against *P. falciparum* malaria by sickle cell haemoglobin.

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PASVOL, WEATHERALL AND WILSON REPLY—Laser and Klein might have been less startled by the results of their analysis of our data[1] if they had considered more carefully the haematology of sickle cell anaemia; taken into account our previous work which shows that *Plasmodium falciparum* preferentially invades young, metabolically active red cells[2,3]; and realised that the data in Table 1 of our paper deal only with parasite invasion and say nothing about development. The red cells in sickle cell anaemia have a short survival time which is reflected by a marked reticulocytosis[4]. Therefore, it is only to be expected that the degree of invasion of sickle cell anaemia (SS) cells by *P. falciparum* would be considerably greater than that of sickle cell trait (AS) or normal (AA) cells. For this reason the only valid comparisons are between cells of like ages (SS with SS, AA with AA) maintained aerobically or under reduced oxygen tensions. The data in Table 1 simply show that cells containing a significant amount of Hb S, such as SS, are invaded less actively under reduced oxygen tensions. However, we are grateful to Laser and Klein for restating that young red cell populations are preferentially invaded by *P. falciparum*.

The main emphasis of our paper was on parasite development once inside the red cells (Tables 3 and 4)[1]. There is no doubt that parasites in cells containing Hb S failed to develop, but only in conditions of reduced oxygen tensions. Furthermore, sickling was not required for this to occur. Laser and Klein are quite incorrect in their assumption that the abnormal forms of the parasite maintained in low oxygen conditions represent the growth of the parasite to a stage at which no further development was possible. Rather, they represent degenerate parasites at early stages of development. This was determined both morphologically and by analysis of pigment content. Furthermore, smears prepared at an early stage in the cultures showed that these abnormal forms were already present. Even if these were entrapped mature forms, which they were not, it is difficult to understand the marked differences observed between the aerobic and low oxygen tension cultures. The growth scores used were simply a means of condensing the data for a large number of experiments. Because the abnormal forms were mainly rings which had failed to develop, it seemed logical to rate them at less than the score of 1 designated to rings. Indeed, the results of the typical experiments in Tables 3 and 4 (ref. 1) stand without the need for growth score analysis.

Laser and Klein's hypothesis that Hb S has a greater buffering capacity than Hb A, thereby reducing fatty acid-induced haemolysis and impeding merozoite release from red cells[5] is interesting. However, at the stage of development before merozoites are released, over 75% of the haemoglobin has been digested by the parasite[6]. Indeed, on this basis it could be argued that relatively increased sensitivity of Hb A-containing cells to lysis might result in premature death and hence offer relative protection to normal individuals with malaria! Furthermore, Laser and Klein's hypothesis is quite incompatible with the mass of epidemiological data suggesting that protection of sickle cell heterozygotes is primarily against *P. falciparum* and not against all species of malarial parasites[7]. Our hypothesis takes into account the behaviour of the mature forms of *P. falciparum* which lodge in deep tissues where the oxygen tissues are low[8] and similar to those used in our experiments[9]. The tendency of Hb S to sickle under reduced oxygen tensions seems more rational than a subtle buffering effect of Hb S as yet not clearly defined in *P. falciparum* infections.

Thus, we believe that our findings, taken together with the essentially similar results of Friedman, who used an entirely different culture system[10], indicate that the growth of *P. falciparum* is limited in cells containing HB S exposed to reduced oxygen tensions and hence provide a reasonable explanation for the protective effect of Hb S against *P. falciparum* malaria.

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