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A Model of How the Sickle-cell Gene Produces Malaria Resistance

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A model of resistance to falciparum malaria provided by the sickle-cell gene in heterozygous state is presented based on earlier hypotheses, the life cycle of *P. falciparum* within its human host, and the nature of host response. Falciparum populations are reduced in size with each cycle of erythrocytic schizogony in sicklers because many parasitized cells are sequestered, sickled, and phagocytized within areas of low P_{O_2} following host vasoconstriction induced by antigen release during schizogony. Populations highly synchronous in their timing of schizogony grow more rapidly than asynchronous populations because a smaller proportion of their members will be trapped, sickled, and phagocytized following vasoconstriction. Differences in synchronicity of schizogony, combined with survival of early ring forms and differences between hosts in time of onset of vasoconstriction, can account for differences in the height of parasitemia between infected sicklers. The sickle-cell gene provides resistance only to falciparum malaria because other forms undergo schizogony in the peripheral circulation where P_{O_2} is too high to allow sickling, and/or their synchrony of schizogony is too great to allow a sufficient proportion of their infecting populations to be destroyed with each cycle of erythrocytic schizogony. Resistance to falciparum malaria based upon the sickle-cell gene is restricted to early childhood because internal organs atrophy consequent to successive sickling episodes brought on by febrile diseases, including falciparum malaria itself. The gradually acquired immunity of both non-sicklers and sicklers reduces the differences in resistance between them until sicklers enjoy no advantage in falciparum resistance. Ways of testing the model are considered.

Since the idea that the sickle-cell gene in heterozygous state provides resistance to falciparum malaria was proposed (Brain, 1952; Mackey & Vivarelli, 1954), and substantiated by field studies (Allison, 1954), the question of how resistance is provided has been open. I here present a model to

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account for resistance based upon a consideration of earlier hypotheses, the life cycle of *Plasmodium falciparum* within its human host, and the response of its host to infection.

A model explaining resistance must account for the following phenomena. (1) Resistance is restricted to falciparum malaria (Allison, 1954). (2) Resistance is only partial and variable in effectiveness. Persons heterozygous for the sickle-cell gene (hereafter called "sicklers") have been observed with falciparum malaria but infections are usually mild, or the frequency of infected sicklers is less than expected (Allison, 1954; Deliyannis & Tavlarakis, 1955; Vandepitte & Delaisse, 1957; Raper, 1959; Brew & Edington, 1965; Edington & Watson-Williams, 1965; Monekosso & Ibiama, 1966). But the level of parasitemia is sometimes as great among sicklers as persons without the sickle-cell gene (hereafter called "non-sicklers"), and may persist long enough to produce gametocytes (Edington & Watson-Williams, 1965; Raper, 1959). (3) Resistance is only effective in early childhood. There are statistically significant differences in resistance between sicklers and non-sicklers when very young (Raper, 1959; Vandepitte & Delaisse, 1957) but only equivocal differences in resistance between adult sicklers and non-sicklers never before exposed to malaria (Buetler, Dern & Flanagen, 1955). (4) Resistance is correlated with the presence of hemoglobin S (hereafter "Hb S"), the product of the sickle-cell gene. No resistance is present until hemoglobin F has been replaced by Hb S (Raper, 1959). (5) Resistance is related to the sickling of erythrocytes. The importance of sickling is implied by: (a) increased rate of sickling with falciparum malaria, as indicated by the forestalling of hemolytic crises by administration of chloroquine to persons homozygous for the sickle-cell gene (hereafter called "sickle-cell anemics") living in malarious areas (Warley *et al.*, 1965); the difference between sicklers, and sickle-cell anemics is one of degree, not kind (Sherman, 1940); (b) increased erythrocyte mortality because of sickling, as indicated by increased erythrocyte longevity following administration of sickling inhibitors (Hathorn & Lewis, 1966); this is important because it will inevitably produce increased mortality of erythrocyte-borne parasites, a crucial result since there is no evidence for differential mortality of merozoites between sicklers and non-sicklers; and (c) probable identification of parasitzed cells by phagocytes which attack sickled cells (Luzatto, Nwachuky-Jarrett & Reddy, 1970) but otherwise cannot tell parasitized from unpararitized cells (Trubowitz & Masek, 1968); parasitized cells sickle 2–8 times faster than unparasitized cells *in vitro* (Luzatto *et al.*, 1970). (6) Resistance is related to sequestration of erythrocytes. Since neither sickling itself, nor sickling crises in sickle-cell anemics occur unless erythrocytes are retained in areas of low P_{O_2} for abnormally long periods (Rucknagel & Neel, 1961),

sickling probably cannot occur rapidly enough to limit parasite populations unless parasitized erythrocytes are sequestered in areas of low P_{O_2}.

To account for all these phenomena, my model incorporates elements of earlier hypotheses and adds four previously ignored elements: internal schizogony, vasoconstriction, synchrony of schizogony, and gradual atrophy of sites of sequestration.

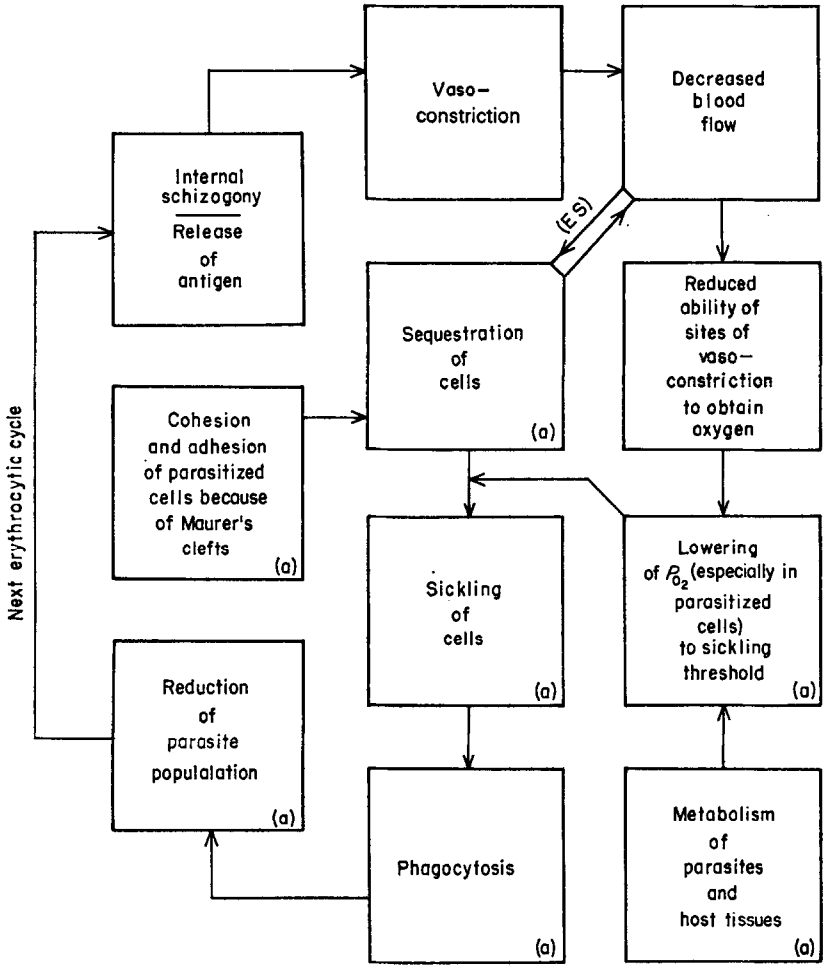

FIG. 1. Flow diagram of events in each cycle of erythrocytic schizogony leading to resistance to falciparum malaria in sicklers. (a) in lower right corners of boxes indicates elements derived from Miller *et al.* (1956). ES is the escape of highly synchronous falciparum populations from the positive feedback loop leading to their extinction in a host (see text).

Figure 1 shows the events occurring in each cycle of erythrocytic schizogony. Events spanning more than one cycle are described in the text. Description begins with the box in the upper left hand corner of Fig. 1. (1) *Plasmodium falciparum* undergoes schizogony in the internal organs of its host leading to lysing of erythrocytes, and the release of merozoites and antigens into the host circulation, and thus stimulates host vasoconstriction (Garnham, 1966). (2) Decreased blood flow consequent to vasoconstriction will sequester many cells in internal organs where they will begin to sickle, especially in the spleen (Hendrickse, 1965; Warley *et al.*, 1965; Cockshott, 1965). Some cells will be sequestered by clumping together and sedimenting as a result of parasite caused surface injuries called Maurer's Clefts (Miller, Neel & Livingstone, 1956). (3) Sickling is promoted by reduced P_{O_2} consequent to reduced inflow of oxygen, and aerobic metabolism of parasites and host tissues (Garnham, 1966). Parasite metabolism tends to make parasitized cells sickle before other cells (Luzatto *et al.*, 1970). (4) Sequestration of cells will itself decrease blood flow, thereby producing a positive feedback loop in which sequestration of some cells leads to sequestration of others, and thus to sickling (Miller *et al.*, 1956; Jandl, Simmons & Castle, 1961). (5) Once sickled, parasitized cells can be preferentially phagocytized, and the falciparum population is thus reduced with every cycle of erythrocytic schizogony (Miller *et al.*, 1956). (6) The ultimate fate of the falciparum population depends on the synchrony of schizogony, and the proportion of parasitized cells sickled and phagocytized. The greater the synchrony of schizogony, the greater the survival rate of the parasite population, for when a high proportion of parasites are released as merozoites nearly simultaneously, only a small fraction of the population will be trapped, sickled and phagocytized following vasoconstriction. Highly synchronous infections may thus grow at or near the rate found in non-sicklers, producing an equivalent pathology. But if merozoite release is asynchronous, a larger proportion of the parasite population will still be bound in cells, and thus be liable to phagocytosis following vasoconstriction and sickling. Asynchronous populations can thus be expected to proceed to extinction within the host, or if they persist, to grow more slowly than those in non-sicklers. Depending on the synchrony of their parasite populations, infected sicklers could appear asymptomatic, or exhibit extreme pathology, including death. Synchrony of reproduction of prey organisms is known to be an important means of reducing the probability of offspring loss to predators, e.g. many East African ungulates sate their dependent carnivores (lions, hyenas, etc.) by calving synchronously (Schaller, 1972). Viewing synchrony of schizogony as a special case of widely occurring synchrony of reproduction is inherently reasonable. (7) Resistance to falciparum malaria is restricted to early child-

hood because internal organs, particularly the spleen, atrophy consequent to hemolytic crises brought on by febrile diseases, including malaria (Sprague & Paterson, 1958; Reynolds, 1965). Atrophy is apparently caused by local necrosis of tissues from oxygen lack due to sequestration of erythrocytes. Organ atrophy could combine with the compensating acquired immunity of both non-sicklers and sicklers to reduce the differences between the two groups with age.

The model can account for all listed phenomena. Restriction of resistance to falciparum malaria results from the internal and usually asynchronous schizogony of *P. falciparum* while other malarias have higher proportions of their schizonts rupturing in the peripheral circulation, and greater synchrony of schizogony (Garnham, 1966). Internal schizogony is critical because oxygen tensions in the peripheral circulation will usually be too high to allow sickling (Rucknagel & Neel, 1961; Henderson & Potts, 1962). The importance of synchrony of schizogony is shown by the high incidence of exposed sicklers infected with *P. malariae* (Allison, 1957), a highly synchronous parasite as indicated by the extremely regular paroxysms it produces (French, 1936).

Partial and variable effectiveness of resistance results from differences in synchrony of schizogony, survival of early ring forms of *P. falciparum* in the peripheral circulation (Garnham, 1966), and time of onset of vasoconstriction between cases. Survival of early ring forms would allow some parasites to persist in asynchronous infections. Variation between hosts in response to antigen release at schizogony would result in some victims vasoconstricting early in an infection and others later, thus some parasite populations would be quashed while small but others only when large.

Restriction of effective resistance to early childhood results from internal organ atrophy consequent to earlier sickling episodes, including previous malaria infections, as noted above.

HbS, sickling, and sequestration of erythrocytes are all related to resistance because the molecular structure of HbS makes sickling possible (Perutz & Mitchison, 1950; Pauling, Itano, Singer & Wells, 1949), sickling identifies parasitized cells to phagocytes, and sequestration is necessary to induce sickling. Although many elements of this model have been presented previously by other workers, no earlier hypothesis has incorporated all of them, and thus no earlier hypothesis has equivalent power of explanation. Allison (1957) tried to account for specificity of resistance by positing HbS was an unsuitable nutrient for *P. falciparum* but not for other plasmodia. Raper (1959) disproved this *in vitro:* moreover, were it true, sickle-cell anemics would never get faciparum malaria because they have no normal hemoglobin (HbA) on which their parasites could feed. But

falciparum malaria has been recorded in sickle-cell anemics (Hendrickse, 1965).

Mackey & Vivarelli (1954) postulated that parasitization enhances sickling, which it does (Luzatto *et al.*, 1970). But sickling alone cannot wholly account for resistance because it does not explain the specificity of resistance to *P. falciparum*, the partial and variable nature of resistance, or the restriction of resistance to early childhood.

Miller *et al.* (1956) proposed seven of the 12 elements in Fig. 1, and thus accounted for more of the dynamism of resistance than other workers. But by ignoring the importance of internal schizogony, vasoconstriction, synchrony of schizogony, and gradual infection-caused organ atrophy, their hypothesis does not account for the partial and variable character of resistance, or its restriction to early childhood. Cohesion and adhesion of parasitized cells because of Maurer's Clefts might restrict resistance to *P. falciparum* since other plasmodia do not produce equivalent cell damage (Garnham, 1966), but they would also lead to a level of parasitemia limited at that population density where infected cells were sequestered at the same rate erythrocytes were newly infected by merozoites. Such a self-limiting level of parasitemia should be fairly constant between cases, and thus there should be little variability in symptoms between patients, a prediction not borne out (Edington & Watson-Williams, 1965; Raper, 1959). Even differences in inocula would only affect the time needed to reach the self-limiting level of parasitemia but not the level itself. Since Maurer's Clefts are a product of the parasite, not the age of the host, cohesion and adhesion of parasitized cells could not account for declining resistance with increasing age of host.

It would be interesting to test my model to see how robust it is in practice. I have not tested it because medical experimentation is outside my province but the kinds of tests needed are straightforward and easily indicated. (1) If internal schizogony is crucial, then parasite populations with higher proportions of their schizonts rupturing in the peripheral circulation should grow more than populations more characterized by internal schizogony. (2) If vasoconstriction is crucial, then (a) cases in which vasoconstriction does not naturally occur with every cycle of schizogony can be expected to exhibit higher levels of parasitemia than those in which vasoconstriction occurs with every cycle; (b) any artificially induced inhibition of vasoconstriction should produce higher levels of parasitemia than would occur without such inhibition; and (c) any artificially induced vasoconstriction before schizogony should reduce the parasite population. (3) If synchrony of schizogony is crucial, then the height of parasitemia in infected sicklers should be a direct function of the degree of synchrony. (4) If sequestration is

crucial, then any inhibition of sequestration should produce a larger parasite population. In practice, this may be the same as the test for vasoconstriction. (5) If sickling is crucial, then any artificial inhibition of sickling, such as by phenothiazine derivatives (see Hathorn & Lewis, 1966), should also produce a larger parasite population.

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