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# Longitudinal studies on human schistosomiasis

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A major difficulty in understanding the epidemiology of human schistosomiasis has been to distinguish between acquired immunity and reduced exposure as possible reasons for an observed decline, in older individuals, of levels of superinfection or of reinfection after chemotherapy. A series of studies of *Schistosoma mansoni* infections in Kenya has been undertaken to approach this problem, by investigation of intensities of reinfection after treatment of individuals whose levels of contact with contaminated water is subsequently observed.

Intensities of reinfection are highest among younger children, thereafter declining sharply. This decline can be attributed only in part to age-related changes in the duration and nature of exposure; there is also evidence for the development of an acquired resistance to reinfection that is dependent both on age and on previous experience of infection, and that may be immunologically mediated. Evidence has been obtained that the slow development of this acquired immunity with age may be associated with the early development and subsequent slow decline of inappropriate immune responses that 'block' the effect of potentially protective responses. Implications of these findings for immunological intervention through vaccination are discussed.

## 1. Introduction

Like many other parasitic helminths, adult schistosomes have a prolonged existence within their definitive hosts, with estimates of mean lifespan in man of *Schistosoma mansoni* and *S. haematobium* ranging from 3 to 6 years (Hairston 1965*a*, *b*; Vermund *et al.* 1983; Goddard & Jordan 1980; Wilkins *et al.* 1984). In various permissive experimental hosts, an acquired resistance to reinfection may develop in spite of the continued presence of adult worms from primary infections, a situation referred to as 'concomitant immunity' (Smithers & Terry 1969). Although, in some experimental models, this resistance may be a non-specific consequence of the elimination of challenge larvae at sites of egg-induced pathology (see Dean (1983)), there is also evidence in many cases for the expression of specific immune responses active against the larvae at some stage during their migration to or maturation in the hepatic portal system (see Capron *et al.* (1982); Capron & Capron (1986)). Adult worms evade immune attack either by acquiring a coating of host macromolecules that mask the parasite's own surface antigens (Smithers *et al.* 1969) or by reducing the expression of antigens in the outer of the two lipid bilayers that make up their tegumental surface (Butterworth *et al.* 1982).

For many years, it was tacitly assumed that the distribution in man of the prevalence or intensity of schistosome infection by age, with a characteristic rapid rise during the first two



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decades of life followed by a progressive slow decline, was attributable to the slow spontaneous death of adult worms from early infections together with a slowly-acquired immunity to reinfection (Gerber 1952; Pesigan *et al.* 1958; Morley-Smith & Gelfand 1960). More recently, however, Warren (1973) has argued that the distribution curves may be explained by the same slow death of adult worms together with reduced exposure to contaminated water in older individuals, and some support for this argument was provided by the observations of Dalton & Pole (1978) that the distribution of intensity of *S. haematobium* infections by age in a Ghanaian community corresponded approximately to the distribution of water contact. However, the analysis and interpretation of the data was by no means conclusive, and failed to resolve the problem of whether or not acquired immunity plays any part in limiting infection among older individuals (Barbour 1985).

Apart from its intrinsic interest, the possible existence of acquired immunity to schistosome infection in man also has practical implications that have recently assumed considerable importance in control. First, the introduction of safe and effective chemotherapeutic agents has shifted emphasis towards chemotherapy as the most favoured method for the control of transmission and morbidity (Cook 1988). However, chemotherapy programmes, at best, achieve control rather than eradication; long-term surveillance and retreatment will therefore always be required, and it is important to be able to identify and focus on those age-groups who have failed to develop an acquired immunity and who therefore remain susceptible to reinfection after treatment. At present, because of the costs of drugs and their delivery, there is a shift away from the mass treatment of all infected individuals towards the 'targeted' treatment of selected groups (Warren & Mahmoud 1980). Until now, however, such targeting has usually been undertaken in an empirical fashion (Mahmoud *et al.* 1983); more effective targeting will depend on the identification, for follow-up after treatment, of susceptible (non-immune) individuals.

Secondly, there is currently considerable interest in the development of recombinant peptides as candidate vaccine antigens. One such antigen, p28, has been shown to be highly effective in eliciting protection in rodent models (Balloul *et al.* 1987), and initial primate experiments have been undertaken (A. Capron *et al.*, unpublished observations). Other candidate antigens are at an earlier stage of development in different laboratories. However, the successful production and delivery of a human vaccine will require considerably more information than has been available until now about the nature and mechanisms of protective immunity in man. In particular, there will be a need to know the extent of immunity that is required to achieve a useful reduction in transmission and morbidity; the existence of any genetic or other predispositions that will restrict the induction of a vaccine-induced immunity; and the nature of the protective response that is required. In this context, evidence is presented below that the isotype of the antibody response to various schistosome antigens governs, at least partly, the expression of immunity; a useful vaccination procedure will therefore depend not only on producing a 'protective' recombinant peptide antigen, but also on administering such an antigen in a way that selects for the most appropriate immune response.

There is thus practical as well as theoretical interest in determining the existence and mechanisms of acquired immunity to schistosome infection in man. This article summarizes briefly the early evidence that suggested the existence of immunity; describes in detail a 'treatment and reinfection' approach to the study of immunity, adopted for both *S. mansoni* in Kenya (Butterworth *et al.* 1984, 1985, 1987, 1988) and for *S. haematobium* in The Gambia



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(Hagan *et al.* 1985, 1987; Wilkins *et al.* 1987); and offers some indication of the factors that may limit the expression of immunity in young children. These studies are not definitive or complete; emphasis is placed on the problems that must be faced, rather than their solution.

## 2. Early evidence for acquired immunity in man

Simple inspection of age-specific prevalence of intensity curves fails to distinguish between acquired immunity and reduced exposure as possible reasons for the progressive reduction in infection in older individuals. Experimental exposure to test directly for the presence of immunity has been carried out in the past (Fisher 1934), but such studies were inconclusive and would now be unacceptable. However, over the course of years, studies of selected communities or groups of patients have provided some evidence that acquired immunity may play a significant role in limiting superinfection (in the absence of treatment) in older individuals.

Several workers have noted that the rate of decline in older individuals of prevalence or intensity of infection is earlier and more rapid in areas of high transmission than in those of low transmission (see, for example, Clarke 1966), observations drawn together by Anderson (1988). As there is no reason to suppose that water-contact levels should decline at an earlier age in areas of high transmission, this rapid decline in egg output suggests the more rapid development of immunity in such areas, the most reasonable interpretation being an earlier and more extensive exposure to schistosome antigens.

Along different lines, Kloetzel & da Silva (1967) compared prevalence and intensities of infection among immigrant communities with those of residents in endemic areas. Among the immigrants, the convex shape of the age-intensity and age-prevalence curves was related to duration of residence within the endemic area, rather than to age as such. As patterns of water contact are strongly related to age (see below), this suggests that the late decline in prevalence and intensity is not solely related to water contact, but instead depends on the duration of previous experience of infection, and may therefore reflect some form of acquired resistance. This type of study is a potentially powerful way of discriminating between purely age-dependent effects (such as changes in water-contact behaviour, or physiological changes) and those associated with previous experience of infection; it has not, however, been repeated in any formal way.

Most of these studies have been cross-sectional in nature. However, there are also a few reports of an apparent acquired resistance based on longitudinal studies of individuals presumed to be exposed to continued infection. Some of these have been purely anecdotal in nature, describing a lack of infection among individuals assumed to be heavily exposed, such as canal cleaners or fishermen (World Health Organization 1974). However, Bradley & McCullough (1973) demonstrated over a three-year period a stability in the relative ranking of egg output in a cohort of schoolchildren infected with *S. haematobium* and suggested that this stability might be attributable to the expression of immunity in some individuals, although this interpretation was subsequently challenged (Jordan *et al.* 1974).

## 3. Treatment and reinfection studies: immunity or exposure?

Although they are strongly suggestive of the development of immunity, four main problems still limit the interpretation of the results summarized in the preceding section.

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First, in the continued presence of adult worms from early infections dying at an unknown and possibly variable rate, it is difficult to determine the extent of new infection that may be occurring, and hence the degree of immunity to these new infections. One approach to this problem, adopted by Wilkins *et al.* (1984), involved a longitudinal comparison of *S. haematobium* infection in two Gambian villages. In one transmission was interrupted by molluscicide, whereas in the second no intervention was undertaken. Observations of the changes with time in intensities of infection in the first village allowed the calculation of an approximate mean lifespan of the adult worm of 3.4 years. Application of this figure to egg counts for the second village allowed the authors to estimate the extent of new infections that were occurring over the three-year follow-up period. The significant feature was that the intensity of new infections in adults was one thousandth of that in young children. Although direct observations of water contact were not made, this difference was considered to be far greater than could be attributed to differences in exposure between the age groups.

An alternative approach to the problem of the continued presence of adult worms, described below, is to examine the rate of reinfection following chemotherapeutic cure of individuals bearing a primary infection. This approach has the obvious disadvantage that chemotherapy itself may affect the immune status of the individual, and therefore that the results will not necessarily reflect the situation in the infected individual. However, it is possible subsequently to identify differences in relevant immune responses between infected and treated individuals respectively. As chemotherapy is at present a major method of control, studies on the effects of treatment on the expression of immunity are themselves of intrinsic interest.

A second problem is the difficulty in determining the relative contributions of acquired immunity and reduced exposure as factors that may limit the levels of superinfection or of reinfection after treatment in older individuals. One possible solution to this problem, mentioned above, is to examine distributions of infection among immigrant populations, in whom previous experience of infection (and therefore probably the development of immunity) is not related to age *per se*. Such communities, however, are difficult to find, and the studies of Kloetzel & da Silva (1967) remain the only good examples. In addition, the development of immunity may well depend not only on previous experience of infection, but also on other factors that remain age-dependent even in immigrant populations. These might include, for example, the phenotypic maturation of the isotype response; previous exposure to ubiquitous cross-reacting antigens; and the changes in antigen experience (for example, in intestinal flora) that occur during physiological maturation, especially at puberty. The only satisfactory solution to this problem, therefore, is to attempt to control for exposure by direct observation of patterns of water contact with known infective sites. This itself engenders problems in determining both the precision and the accuracy of the observations that are made. Some approaches to this are outlined below.

Thirdly, many previous studies have relied solely on analysis of incidence or prevalence of infection, rather than intensity. In the various animal models, whether of natural infection or of vaccine-induced resistance, immunity is rarely complete; a proportion of larvae of a challenge infection invariably mature and lay eggs, even though that proportion is considerably less in immune than in susceptible animals. There is no reason to suppose that humans should differ in this respect. However, an incomplete immunity may be of considerable value in limiting not only transmission but also disease. It is generally accepted that the extent of disease is dependent on the numbers of eggs that are deposited in the tissues, and hence on the



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numbers of adult worms harboured by the host (Hiatt 1976; Siongok *et al.* 1976; the distribution of intensity of infection is overdispersed (Anderson 1988), and severe disease develops primarily in the small numbers of individuals who bear heavy worm burdens. Therefore, any event – such as an incomplete immunity – that even partly reduces the establishment of adult worms may have a considerable impact on the prevalence of disease, by reducing the numbers of individuals in the high-intensity category. It is important, therefore, to be able to detect an incomplete immunity. This requires repeated, quantitative measurements of intensities of superinfection, or of reinfection after treatment, rather than simple assessment of the presence or absence of infection.

Finally, the aggregated distribution of worm burdens in individual communities suggests a marked heterogeneity, at the individual level, in either exposure or susceptibility to infection or both. A heterogeneity in susceptibility could be attributable to a genetic predisposition – an obvious example being an MHC restriction in immune responsiveness, already demonstrated in the context of egg-induced pathology (Hirayama *et al.* 1987) – or to a variety of phenotypic factors including nutritional status, previous exposure to cross-reactive antigens or the presence of concurrent, immunosuppressive infections. The nature of such factors will be difficult to resolve; the important point, however, is that during the early stages of a study any analysis should take into account the possibility of a very high level of individual variation in the level of expression of a partial immunity.

In several recent studies attempts to overcome some of these problems have been made by undertaking repeated, quantitative observations of the intensities of reinfection after chemotherapeutic cure of individuals whose levels of exposure to contaminated water is subsequently observed. These are long-term studies, and results from Brazil by Gazzinelli and colleagues and Dessein and colleagues are not yet published; this section concentrates on the recent findings of Wilkins and colleagues for *S. haematobium* in The Gambia and on our own work on *S. mansoni* in Kenya. Both of these studies have yielded evidence for the slow development with age of an acquired resistance to reinfection after treatment, and have provided pointers towards some associated immunological mechanisms.

### *S. haematobium in The Gambia*

Two studies of *S. haematobium* infections have involved detailed observations of exposure and of reinfection intensities following treatment, together with a variety of immunological investigations. In both, estimates of exposure were based on the observed duration of contact, corrected for the extent of body contact and the infectivity of the water, as judged by snail infection rates and cercarial densities. The first comprised a group of 50 children, aged 8–13, in whom it could be shown that the presence of reinfection 15 months after successful chemotherapy was significantly associated not only with exposure but also with the absence of elevated peripheral blood eosinophil counts (Hagan *et al.* 1985, 1987). These findings, discussed below, suggested that resistance to reinfection might be immunologically mediated and associated with eosinophil-dependent effector mechanisms, although antibodies mediating eosinophil-dependent killing of schistosomula were not detectable in these children.

In this study both age and exposure, as well as eosinophil counts, were significant explanatory variables. In a second study of a cohort of 107 individuals of all ages further evidence was again obtained for a marked effect of age, separable from age-dependent changes in the levels of exposure (Wilkins *et al.* 1987). Intensities of reinfection 15 months after

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treatment were significantly related both to exposure and to age and, when exposure levels were stratified, it was found that a category of heavily-exposed children under the age of 9 showed intensities of reinfection more than 100 times those of comparably exposed females over the age of 15. Thus although levels of exposure were generally lower in the older age-groups those adults who were heavily exposed showed clear evidence of a marked, age-dependent, acquired resistance to reinfection.

## *S. mansoni in Kenya*

### *Study design*

In parallel with the study on Gambian children described above, a pilot treatment and reinfection study of *S. mansoni* infections in Kenya by Sturrock *et al.* (1983) demonstrated a significant association between high circulating eosinophil counts, the presence of antibodies mediating eosinophil-dependent killing of schistosomula and low intensities of reinfection following treatment of a group of 489 children. In this study, direct observations of water contact were not made. However, it was also noted that low intensities of reinfection were associated with age and with the distance of the children's households from the source of infection. These findings justified a more extensive and detailed investigation, in which parallel observations would be made of water-contact behaviour, reinfection intensities following treatment, and various immune responses. These studies, which started in 1980, are still in progress, and have been divided into three phases.

During the first phase, from 1980 to 1983, observations were made in the village of Iietune, a community of some 1600 people in Machakos District that shows a typical picture of high transmission of *S. mansoni* within a subsistence agricultural economy. The climate is generally dry, and water supplies are limited to a single small permanent stream, together with a few semi-permanent pools in otherwise dry watercourses. This allowed the identification and regular observation of the limited number of water-contact sites frequented by members of the community, these sites also being regularly sampled for the presence of infected snails (Butterworth *et al.* 1984). Stool samples were also collected at periodic intervals either from the entire community or from the children attending the local primary school, and were examined for *S. mansoni* infection by the quantitative Kato technique (Sturrock *et al.* 1983). After a year of baseline observations, a group of 129 children from the primary school, aged 9–16, were treated and followed for intensities of reinfection at three-monthly intervals over the subsequent 21 months. Blood samples were taken from these children before treatment and at regular intervals thereafter for investigation in a range of immunological assays, described below.

At the end of this phase, in October 1983, treatment was offered to all infected individuals in the Iietune community. At this stage, no intervention was made in the surrounding communities, whose members also frequented the waterbodies used by the Iietune residents. In consequence, transmission continued in Iietune at high levels during the period to August 1984. This allowed investigation, on a community basis, of the incidence and intensities of reinfection during the nine months following treatment, and of new infections among previously uninfected and untreated individuals, which could be related to levels of water contact.

Subsequently, the study has been expanded into a comparative chemotherapy programme comprising approximately 13000 people in four main treatment groups and their respective



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buffer zones. The aim of this programme is to compare the cost and effectiveness in reducing transmission and morbidity of:

(i) treatment of all infected individuals;

(ii) treatment targeted towards individuals bearing heavy infections of more than 100 eggs per gram (EPG) of faeces;

(iii) treatment of all infected primary school children;

(iv) treatment within a witness area only of those individuals with severe infections (more than 800 EPG) or clinical morbidity.

The prediction is that, because the schoolchildren include most of the heavily infected individuals within the community, and because they contribute most to contamination by indiscriminate defaecation in and around the water bodies, treatment of children alone will be as effective as treatment of all heavily infected individuals. In addition, it is predicted to be cheaper and easier to administer than the other measures in terms of diagnosis, drug costs, drug delivery and follow-up. Finally, on the basis of results from the earlier phases (see below), it is predicted that reinfection will occur primarily among the young children who remain susceptible, and that surveillance can therefore be limited to this age group.

Following a year of precontrol observations, treatment was delivered in all areas in April 1985. Post-treatment follow-up will continue until 1989, and complete analysis is therefore not yet available. However, preliminary analysis of the stool results for the first two years after treatment supports the hypothesis that treatment only of infected schoolchildren causes a marked and durable reduction in intensities (although not prevalence) of infection, the costs of diagnosis, drugs and their delivery being considerably less than in those areas in which treatment was based at the community level (R. F. Sturrock *et al.*, unpublished observations). Within the expanded study areas, additional cohorts of individuals of all ages have also been selected for detailed immunological and other investigations. However, reinfection data for these study groups are not yet complete, and they are not considered further in this review.

*Reinfection among children*

During the six months following treatment of the 129 children in Iietune in October 1981, there were high levels of transmission in the study area (Butterworth *et al.* 1985). Over this period most of the treated children became reinfected, implying that any immunity that may have been present was incomplete, as it is in the various animal models. However, the mean intensities of reinfection were tenfold lower than the pretreatment levels, and these intensities remained stable throughout the remainder of the 21 month follow-up. In addition, a marked variation was observed in the intensities of reinfection between different individuals. This allowed the initial characterization at a simple level of two extreme subgroups: those showing high intensities of reinfection, and therefore susceptible, and those showing low intensities of reinfection in spite of equally high levels of water contact, and therefore at least partly resistant. These two groups showed no difference in mean pretreatment intensity of infection, but did differ markedly in age, the mean age of the resistant children being 2.0 years greater than that of the susceptible children, within a restricted starting-age range. This provided initial evidence, therefore, for an acquired and age-dependent resistance to reinfection, independent of levels of water contact; a possible immunological basis for the age-dependent effect among these children is described below.

Subsequently analysis, focusing on the variation between individuals for the study group as



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a whole, revealed two points. First, there was a marked negative rank correlation between reinfection intensity and age. In addition, although water contact levels were positively correlated with pre- or post-treatment intensity of infection (indicating that observations of water contact can provide an estimate of exposure), there was no negative correlation between age and water contact levels within this limited age range. The hypothesis that the negative correlation of reinfection with age was attributable to age-dependent changes in exposure was therefore not supported. Secondly, there was evidence for an individual predisposition to heavy infection; that is, there was a significant rank correlation between pre- and post-treatment intensities (Bensted-Smith *et al.* 1987). This effect was more marked among the younger children; the smaller effect among older children may reflect the increasing importance with age of acquired resistance, as opposed to predispositional effects, in limiting reinfection. The nature of the predisposition, whether genetic or behavioural, is not demonstrated by this analysis. Its potential significance is that a predisposition to heavy infections may enhance the impact of targeted chemotherapy programmes (Anderson & May 1982, 1985; Anderson & Medley 1985), at the same time rendering easier the selection of patients for surveillance following treatment.

*Reinfection in the community*

Among the small group of primary schoolchildren described above, there was no evidence that age-dependent changes in water contact contributed to the observed decline with age in intensities of reinfection after treatment. However, when the entire Iietune community was treated and subsequently examined for reinfection nine months later, there was clear evidence for changes with age in water contact behaviour. This has complicated the analysis of a possible role of acquired resistance in limiting reinfection over a broad age range; an initial approach is outlined below.

Behavioural factors that may affect the degree of exposure to cercarial invasion include not only the duration of water contact and the numbers of cercariae in the water, but also the extent of contact (in terms of body area exposed) and the nature of contact. For example, five minutes spent washing clothes with soap – which, at high concentrations, is strongly cercaricidal – is likely to be less hazardous than five minutes spent swimming. Any estimate of exposure must therefore take into account not only the duration of contact observed, but also the infectivity of the water at the time of contact and the nature of the contact. All of these variables may interact both with each other and with age, and the effect of each variable on true exposure cannot be estimated with accuracy.

An approach adopted by Wilkins *et al.* (1987) in their studies on *S. haematobium* in The Gambia has been to weight the observed duration of contact for each individual by an arbitrary correction factor based on the one hand on cercariometric observations and on the other hand on the body area exposed during each contact and on the use of soap. Application of this stringent correction factor markedly reduced the levels of exposure calculated for older age groups, especially women, in comparison with the initial observed duration of contact. In spite of this, there was still evidence for a reduction with age in reinfection in heavily exposed individuals. This implied a marked effect of an acquired resistance, the effect being, if anything, underestimated.

A similar approach has been adopted by G. F. Tingley *et al.* (unpublished observations) to the data for those members of the Iietune community who were successfully treated in October



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1983 and were subsequently re-examined in August 1984. Stratification of intensities of reinfection by age showed a progressive increase in young children, rising to a sharp peak in the 9–12-year-olds, with very low levels in older children and adults. In contrast, uncorrected estimates of the duration of water contact revealed a broad peak in adults aged 17–24. When these estimates were weighted for site, season, time of day, nature and extent of contact by factors that reflected the estimated hazard of each exposure, the distribution of reinfection approximated to the distribution of corrected exposure; rank correlations between the two variables were highly significant. However, changes in exposure still appeared insufficient to account for the changing distribution of reinfection with age. For example, individuals aged 21–24 showed over half the level of corrected exposure that was observed in the 9–12-year-olds, but only 1% of the level of reinfection, again implying an effect of a marked, age-dependent resistance superimposed on a relative lack of reinfection attributable to reduced exposure.

A problem with this approach is that the weighting factors used, although selected on biologically meaningful criteria and designed to militate against the detection of an age-dependent acquired resistance, were arbitrary. An alternative approach, therefore, is currently being tested (A. J. C. Fulford *et al.*, unpublished observations). This involves the use of the water contact data themselves to generate a non-linear model that maximizes the likelihood of predicting intensities of infection or of reinfection after treatment. This model takes the form

$$\mathrm{E}\sqrt{Y_i} = \beta_0 + \beta_1 \sum_{j=1}^{n_i} w_{\mathrm{sit}}\, w_{\mathrm{deg}}\, w_{\mathrm{act}}\, w_{\mathrm{tim}}\, w_{\mathrm{mon}}\, w_{\mathrm{age}}\, DUR_{ij},$$

where $\mathrm{E}\sqrt{Y_i}$ = expected $\sqrt{}$(eggs per gram) for $i$th individual; $n_i$ = number of observations for $i$th individual; $DUR_{ij}$ = duration of $j$th observed contact of $i$th individual; $w_{\mathrm{sit}}$ = weighting factor for site of $ij$th observation; $w_{\mathrm{deg}}$ = weighting factor for degree of $ij$th observation; $w_{\mathrm{act}}$ = weighting factor for nature of activity of $ij$th observation; $w_{\mathrm{tim}}$ = weighting factor for time of day of $ij$th observation; $w_{\mathrm{mon}}$ = weighting factor for month of $ij$th observation; and $\beta_0$, $\beta_1$, $w_{\mathrm{sit}}$, $w_{\mathrm{deg}}$, $w_{\mathrm{act}}$, $w_{\mathrm{tim}}$, $w_{\mathrm{mon}}$ and $w_{\mathrm{age}}$ are constants estimated by fitting the model.

The nature of the weighting factors that are generated for behavioural variables may then be examined, and any effect of age may subsequently be tested by inclusion as an additional weighting factor in the model. This approach attempts initially to explain all aspects of reinfection in terms of behaviour, and therefore militates against the detection of additional age-dependent effects. An example of a preliminary application of this model is shown in tables 1 and 2 and figure 1 (for the data for pretreatment intensities of infection in Iietune).

Fitting the model without age (table 1) led to an increase in the value of the correlation coefficient, $r$, from 0.256 to 0.390, with the generation of weighting factors consistent with those predicted from other information. In particular: (*a*) the weighting factors for site were greater for the heavily infested mainstream sites, especially sites 7–18, than for the lightly infested sidestream sites; (*b*) watering vegetables, a common activity involving only intermittent contact with water, was associated with a low weighting factor; (*c*) contacts involving greater body areas, especially whole body contact, were associated with high weighting factors; (*d*) contacts before 10 a.m., the time when cercariae are first shed, were associated with low weighting factors; (*e*) contacts during January and February, and September and October (periods in which, in this particular year, few snails were found), were associated with low weighting factors.

Subsequent inclusion of age in the model (table 2) was associated with an increase in $r$ from



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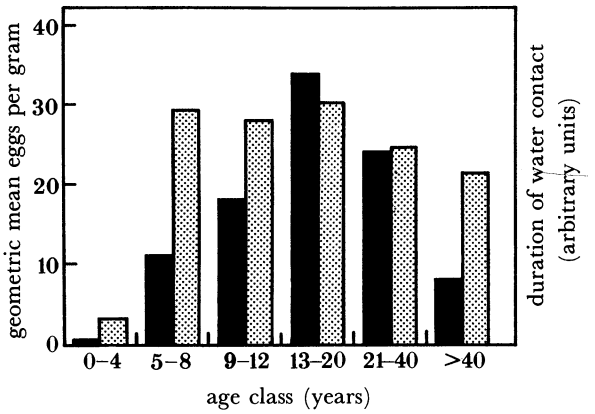


FIGURE 1. Relation between pretreatment intensity of infection (Iietune, August 1983) and weighted water contact levels during the preceding year. ■, Geometric mean EPG; ▨, water contact levels fitted by the model shown in table 1 (excluding age).

TABLE 1. WEIGHTING FACTORS GENERATED BY MAXIMUM LIKELIHOOD FITTING OF A NONLINEAR MODEL AGE (AGE EXCLUDED)

| level | site[a] | activity[b] | degree[c] | hour[d] | month[e] |
|---|---|---|---|---|---|
| 1 | 0.64 | 4.75 | 0.85 | 0.03 | 0.07 |
| 2 | 2.06 | 2.03 | 0.25 | 1.13 | 1.79 |
| 3 | 2.25 | 0.60 | 1.15 | 1.08 | 1.27 |
| 4 | 0.00 | 0.01 | 2.78 | 1.22 | 0.84 |
| 5 | 0.11 | 0.31 | — | 1.57 | 0.01 |
| 6 | 0.14 | 1.47 | — | — | 1.89 |
| 7 | — | 0.56 | — | — | — |

unweighted $r = 0.256$, 1025 d.f.
weighted $r = 0.390$, 1002 d.f.

[a] Site: 1, sites 1–6 (mainstream); 2, sites 7–12 (mainstream); 3, sites 13–18 (mainstream); 4, sites 19–24 (mainstream); 5, site 25 (sidestream); 6, site 26 (sidestream).
[b] Activity: 1, crossing, walking, grazing cattle; 2, drawing water; 3, drinking or unspecified; 4, watering vegetables; 5, bathing or washing body; 6, washing clothes; 7, swimming or playing.
[c] Degree: 1, hands, face or feet; 2, hands+face or hands+feet; 3, hands+face+feet; 4, whole body.
[d] Hour: 1, 06h00 to 10h00; 2, 10h00 to 12h00; 3, 12h00 to 14h00; 4, 14h00 to 16h00; 5, 16h00 to 18h00.
[e] Month: 1, Jan./Feb.; 2, Mar./Apl; 3, May/Jun.; 4, Jly/Aug.; 5, Sep./Oct.; 6, Nov./Dec.

TABLE 2. WEIGHTING FACTORS GENERATED BY MAXIMUM LIKELIHOOD FITTING OF A NONLINEAR MODEL (AGE INCLUDED)

(Levels for site, activity, degree, hour and month as in table 1.)

| level | site | activity | degree | hour | month | age[a] |
|---|---|---|---|---|---|---|
| 1 | 0.74 | 4.82 | 0.77 | 0.01 | 0.07 | 0.92 |
| 2 | 2.01 | 2.33 | 0.25 | 1.64 | 1.78 | 1.46 |
| 3 | 2.14 | 1.27 | 2.62 | 1.09 | 1.45 | 0.69 |
| 4 | 0.00 | 0.11 | 2.91 | 0.98 | 0.53 | — |
| 5 | 0.10 | 0.33 | — | 1.43 | 0.01 | — |
| 6 | 0.07 | 1.01 | — | — | 2.20 | — |
| 7 | — | 0.15 | — | — | — | — |

unweighted $r = 0.256$, 1025 d.f.
weighted $r = 0.408$, 1001 d.f.

[a] Age: 1, less than 9 years; 2, 9–20 years; 3, over 20 years.



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0.256 to 0.408. Values for the weighting factors for the different levels of the other variables were little altered. However, the weighting generated for age was pronounced, the factor for children between 9 and 20 being twice as great as that for adults over the age of 20.

Figure 1 shows the relation between age, egg counts and water contact levels weighted by fitting the model without age. In this case, pretreatment egg counts are recorded, which reflect previous cumulative experience of infection. After the age of 4, children show high levels of exposure (weighted water contact) which are reflected by a progressive increase in egg counts as the children continue to accumulate infections. After the age of 20, however, the pattern is reversed; egg counts progressively and rapidly decline, in spite of the fact that these older individuals continue to show high levels of weighted exposure.

Although these findings support the hypothesis, previously developed for the study group of 129 children, of the development during late childhood of an acquired resistance to reinfection, various problems remain. At present the model, although stable for the pretreatment data, is unstable for the data for reinfection after treatment. It is affected by the order of iteration; it sometimes yields large negative weighting factors unless these are disallowed, and it may give poorer fits with larger numbers of variables. These problems may be due partly to correlations between the covariables, and partly to the skew distribution of the post-treatment data, with large numbers of zero egg-counts. Further work to overcome these problems will test the effect of fitting Poisson rather than normal error structures.

In addition, two other basic problems remain to be resolved. First, it is not possible formally to exclude the possibility that the inaccuracy of water contact observations as a true measurement of exposure may lead to an overestimate of exposure in older individuals. Secondly, the demonstration of an age-dependent effect does not formally distinguish between an age-dependent resistance to reinfection acquired as a result of previous exposure (and therefore reflecting an acquired immunity) and other age-dependent physiological events, such as changes in skin thickness or endocrine changes associated with puberty. To overcome these problems, we shall attempt to use the model described above (once fully developed) to compare intensities of infection in two groups of subjects: those who were infected and successfully treated in October 1983, and those who were not detectably infected and were therefore not treated. By making this comparison, it should be possible to control for the effect of age *per se* in investigating the effect of previous (known) experience of infection on subsequent susceptibility to reinfection. Initial inspection of the raw data (figure 2) reveals a marked

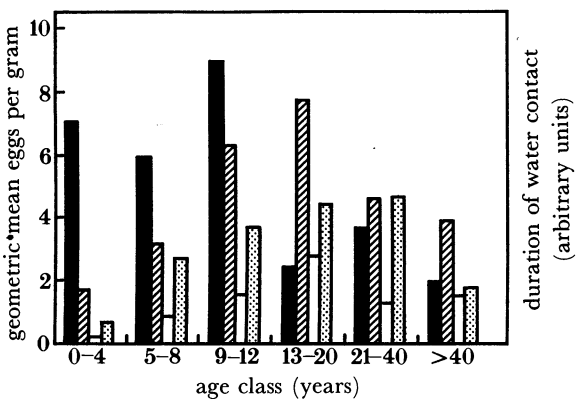


FIGURE 2. Relation between intensity of infection after treatment (Iietune, August 1984) and unweighted water-contact levels during the intervening period. ■, Geometric mean EPG and ▨, unweighted water-contact levels for individuals who were successfully treated in October 1983; □, geometric mean EPG and ▩, unweighted water-contact levels for individuals who were not detectably infected, and were therefore not treated.



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decline in reinfection intensities of previously infected individuals in the age-range 13–20 years, that is not seen in previously uninfected individuals and is not reflected by a similar decline in water-contact levels. The pattern for individuals aged over 20 is less clear, possibly because those categorized as uninfected had previously been infected but had spontaneously lost their infections.

These various findings, although still incomplete and not providing a clear indication of the relative contributions of exposure and of acquired resistance in determining the levels of superinfection or of reinfection after treatment, strongly suggest that both are important factors. Further studies on the original group of 129 children have provided more information about the immunological processes that may be involved.

## Mechanisms of immunity

Studies in experimental animal models of schistosome immunity have demonstrated a range of immune effector mechanisms that can be divided broadly into two categories.

(i) Those that are active against the young schistosomula soon after penetration of the skin. These are primarily antibody-dependent, cell-mediated reactions, involving either IgG or IgE antibodies with specificity for schistosomulum surface antigens, and with eosinophils and macrophages being particularly effective killer cells (see Butterworth 1984).

(ii) Those that are active against older schistosomula, during or even after their migration to the hepatic portal system. These appear not to involve reactions against surface antigens (which are not expressed on older organisms); instead, a nonspecific effect of activated macrophages has been proposed.

However, each experimental model differs quite markedly from the next, and there is no good reason to assume that any mechanism demonstrated in experimental systems will necessarily be operative in man. Instead, studies in humans have relied mainly on the demonstration of immune effector mechanisms *in vitro*, and their subsequent correlation with the expression of immunity. This approach has the obvious danger of revealing significant but non-causal correlations, which may best be avoided by establishing a meaningful pattern of correlations, and by using observed correlations to devise new assays that are predictive of immunity.

The antibody-dependent killing of schistosomula by human eosinophils is more marked when eosinophils are recovered from the blood of individuals with a helminth-induced or other eosinophilia (David *et al.* 1980). Such cells are said to be activated, an effect associated with the production by monocytes from eosinophilic individuals of an eosinophil-activating factor (Thorne *et al.* 1985). If the expression of immunity was dependent on eosinophil-mediated mechanisms, therefore, one might expect a correlation between circulating eosinophil counts (reflecting indirectly the state of eosinophil activation) and resistance to reinfection. Such a correlation was found in a pilot study of *S. mansoni* in Kenya (Sturrock *et al.* 1983), and subsequently in the more detailed study of *S. haematobium* in The Gambia described above (Hagan *et al.* 1985).

However, an extensive series of investigations on the 129 Kenyan schoolchildren treated for *S. mansoni* infections and subsequently followed for reinfection for 21 months, has failed to confirm this correlation. Instead a more complicated picture has emerged that may help to explain the very slow development of immunity in young children in the face of continued exposure to schistosome antigens throughout childhood (Butterworth *et al.* 1985, 1987, 1988; Dunne *et al.* 1987, 1988).



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Two possible explanations for this slow development of immunity have been considered. First, it may be that young children, exposed over long periods but to extremely small levels of 'protective' schistosomulum antigens, simply take a long time to develop protective responses. However, no evidence has been obtained to support this hypothesis. All the children, including the younger ones who remain susceptible to reinfection after treatment, show a range of potentially protective responses, and there is no correlation between such responses and the expression of immunity. These include circulating eosinophil levels, the levels of heat-stable (IgG) antibodies mediating eosinophil-dependent killing of schistosomula and the levels of IgE antibodies with specificity for schistosomulum antigens. In a preliminary study in Egypt, Colley *et al.* (1986) have demonstrated an association between resistance to reinfection after treatment and lymphocyte proliferative responses to cercarial and adult worm antigens (suggesting a protective role of cell-mediated responses). However, recent experiments in Kenya on the newer study groups of all ages have failed to reveal the predicted increase with age in either lymphocyte proliferation or gamma-interferon production (as an indirect assay for macrophage activation) in response to a range of schistosome antigens (A. E. Butterworth, R. K. Gachuhi and J. H. Cuma, unpublished observations).

Instead, evidence has been obtained that the slow development of immunity may be attributable to an early rise and subsequent slow decline of antibodies of an inappropriate isotype that block the expression of concomitant protective responses. This was originally suggested by the finding that the susceptibility of the younger children to reinfection nine months after treatment was positively correlated with high levels, in the preceding blood samples, of anti-egg antibodies, including antibodies that also recognised a carbohydrate epitope expressed on a major 38 kDa schistosomulum surface antigen, as judged by the inhibition of binding of a rat monoclonal antibody (Butterworth *et al.* 1987). This suggested that antibodies of an inappropriate isotype, elicited in response to egg polysaccharide antigens, might cross-react with schistosomulum surface carbohydrate epitopes and block the binding of antibodies of an 'effector' isotype with specificity for the same or closely adjacent epitopes.

Initial support for this hypothesis came from the observation that individual sera contained IgM antibodies that would inhibit the eosinophil-dependent killing of schistosomula mediated by IgG antibodies from the same sera, or by the intact sera themselves (Khalife *et al.* 1986). In addition, it was found that the originally defined subgroup of young, susceptible children had higher levels of IgM antibodies with specificity for schistosomulum surface antigens than the older, resistant children. Subsequently, attention has been paid to the nature of the cross-reactive egg antigens and of the antibodies that they elicit.

Examination of the relation between anti-egg antibody responses and intensities of reinfection revealed a dissociation between the different subclasses (Dunne *et al.* 1988). IgG4 antibodies in the pretreatment blood sample were significantly correlated with pretreatment intensity of infection, and may therefore simply have reflected the extent of exposure to eggs. In contrast, IgM and IgG2 anti-egg antibodies showed no correlation with pretreatment intensities, but did correlate positively with intensities of reinfection nine months after treatment, and may therefore act as blocking antibodies. In addition, it was found that IgG4 antibodies were elicited exclusively in response to glycoprotein components of eggs, whereas IgM and IgG2 were elicited predominantly in response to egg polysaccharides; and the relation with susceptibility was more marked for the anti-polysaccharide response. Subsequently it was found that the IgM and IgG2 antibody responses correlated strongly with responses against carbohydrate components of schistosomulum surface membrane antigens; in



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contrast, IgG4 anti-egg antibodies correlated with responses against the peptide components of the membrane. Finally it was found that there was a positive association between IgM anti-schistosomulum total membrane and shed antigen responses and both pre- and post-treatment intensities of infection. Total IgG responses against the shed antigens showed a negative correlation with reinfection that was more marked when those patients with high levels of cross-reactive IgG2 anti-egg antibodies were excluded (Butterworth *et al.* 1988). These correlations with susceptibility or resistance were most marked for the anti-carbohydrate component of the anti-schistosomulum response, and in particular for the antigens that are shed from the surface of the schistosomulum during incubation *in vitro*. Results for the individual IgG subclasses of the anti-schistosomulum response are not yet available, but the findings to date support the hypothesis that IgM and IgG2 anti-egg polysaccharide antibodies cross-react with schistosomulum surface carbohydrates and block the binding of protective IgG antibodies of another subclass (possibly IgG1) that has the capacity to mediate killing of schistosomula through eosinophils or other effector cells. The IgM and IgG2 antibodies may then decline with age, permitting the expression of the pre-existing protective responses, and hence of immunity.

## Conclusions

The findings summarized here, both of our own work in Kenya and of that of other groups, especially Wilkins and colleagues in The Gambia, offer some explanation of the dynamics of schistosome infections in man and have implications for the development of useful vaccines. Reduced levels of exposure may account in part for the relative lack of superinfection, or of reinfection after treatment, that is observed in older individuals. In addition, however, there is now a substantial body of evidence for the slow development with age of an acquired immunity to reinfection, distinguishable from the effects attributable to altered levels of exposure. The curious feature is that such immunity is very slow to develop (in comparison with immunity against most prokaryotic and protozoan parasites), taking many years of primary infection or of continued exposure to new infections. At least one reason for this slow development of immunity is that, during the early years of childhood infection, the host mounts a series of inappropriate responses that 'block' the effect of protective responses present simultaneously.

This phenomenon may be regarded as being of adaptive advantage to the parasite. Under the conditions of infrequent, trickle infection to which humans are naturally exposed, it is of advantage to the parasite that the host should progressively build up a substantial worm burden for the maintenance of transmission, and therefore that he or she should not rapidly develop an immunity to superinfection. Equally, however, it is of advantage to the parasite that the host should not eventually die as a consequence of the overwhelming worm burden that could result from prolonged exposure, and therefore that he or she should eventually develop an immunity to superinfection. The early rise and subsequent decline of blocking antibodies permits this state of affairs, and is achieved by exploitation of the different kinetics of what might be regarded as T-independent and T-dependent responses to polysaccharides and glycoprotein antigens respectively. It may be that the main evolutionary significance of the polysaccharides that are released in large amounts from eggs is to distract the host's immune system into mounting, at least temporarily, an inappropriate response.

The implications for vaccine development are considerable. The demonstration of significant



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correlations of resistance (or susceptibility) with responses directed against schistosomulum surface antigens indicates that protective responses against such antigens can be effective under conditions of natural exposure, and therefore that they might be suitable for inclusion in a vaccine. Such antigens may include both those that are retained at the surface of the schistosomulum and those that are released during maturation. Although the advent of molecular biological techniques has led to a concentration on peptide antigens, the major role of anti-carbohydrate responses described here suggests that the manipulation of schistosome polysaccharides will also be an area worth pursuing. Of crucial importance, however, whether for peptide or carbohydrate vaccines, will be the design of protocols or adjuvants that will selectively elicit the correct subclass of response; this field is still underdeveloped. Finally, it is unlikely that a vaccine will elicit a sterile and permanent immunity and, although an incomplete immunity will be of value in limiting both transmission and disease, it will be important to learn more about the effects of subsequent, low-level infections on the expression of a vaccine-induced immunity. Such infections could boost existing immunity, or they could lead to the formation of blocking antibodies that will reduce the effects of previous vaccination. Before embarking on large-scale vaccine trials, it would be useful to learn more about the effects of low-level infections in eliciting blocking antibodies, and about the kinetics of the immune response in adults who have not previously been exposed.

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## *Discussion*

D. A. P. Bundy (*Department of Pure and Applied Biology, Imperial College, London, U.K.*). For blocking antibody to play an age-dependent role in determining resistance to schistosome infection, it would be necessary for the levels of the supposed blocking IgM and IgG isotype to decline in an age-dependent manner, in the presence of continuing infection exposure. Does Dr Butterworth have evidence for such a decline and can he suggest a mechanism for this occurrence?

A. E. Butterworth. Yes, both the IgM and IgG2 anti-carbohydrate responses decline with age in the manner described. We have no direct evidence for the mechanism of such a decline: my guess is that they represent T-dependent responses to repeated carbohydrate epitopes. As such, they would show no anamnaestic effect attributable to the continued presence of antigen. In contrast, IgG1 and other T-dependent responses to the same carbohydrate epitopes expressed on glycoproteins may show a conventional boosting effect. However, this is speculative, and we have no direct evidence for the mechanism of the decline in IgM and IgG2. All we can say is that such a decline occurs.



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