A simple model of the build-up of resistance to mixtures of anti-malarial drugs

C. F. CURTIS AND L. N. OTOO*
London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT

Abstract

In an organism with a life-cycle modelled on that of *Plasmodium* two separate resistance genes are assumed, each protecting against one of two unrelated drugs. The model was used to compare the rates of build-up of resistance in a population where the two drugs are used either as a mixture or in sequence. The model suggests that the use of a mixture would be advantageous if: (i) both resistances are initially rare; (ii) there is recombination between the genes; (iii) a large proportion of the parasite population is unexposed to the drugs.

The use of certain mixtures of anti-malarial drugs has been advocated, and there is evidence from selection experiments with syringe-passaged rodent malaria that the use of mixtures is less prone to select double resistance than is the sequential use of the same two drugs (PETERS, 1984; PETERS & ROBINSON, 1984). With some mixtures the components interact and potentiate each other. However, here we wish to analyse the simpler case in which the components do not metabolically interact with each other. In these circumstances the use of a mixture may be advantageous if each resistance gene is rare, because then the double resistant combination would be expected to be extremely rare, assuming random association of the two genes. In populations of limited size the double resistant combination may not exist at all, and this may be the reason for the success of the laboratory experiments in delaying resistance by the use of mixtures.

In large natural populations of *Plasmodium* it would seem unwise to assume that even very improbable gene combinations would be totally absent but, provided that a fraction of the population is unexposed to drug selection, the number of double resistants which selectively survives in treated malaria patients is likely to be small compared with the number of parasites which is unexposed to selection in untreated patients. Table I demonstrates the principle over one round of selection and emphasizes that the mixture only has a significant advantage over a single drug where the resistances to both are rare (in the Table a resistance at a frequency of 0·1% is taken as "rare" in contrast with 50%). PETERS & ROBINSON (1984) found that a mixture was advantageous even when starting from a *Plasmodium* population already resistant to one of the components. This seems to implicate a metabolic interaction between the components of their mixture and, as already stated, we cannot deal with such complications in our simple model.

Examples similar to those in Table I were used to support the concept of using mixtures of insecticides to delay the build-up of resistance to each of the compounds (CURTIS, 1985). However, this study showed that when one considers selection over a series of insect generations there is, in certain circumstances, a problem of selective build-up of non-random association of the two resistance genes (linkage disequilibrium), which could reduce or eliminate the advantage of using a mixture as against using the two compounds sequentially. Genetic recombination tends to randomize the association between the resistance and susceptibility alleles.

In *Plasmodium* the blood stages are now known to be haploid, and there is effective genetic recombination after fertilization in the mosquito stages of the life-cycle (WALLIKER, 1983). A computer model was constructed to investigate the process of prolonged selection for resistance by two anti-malarial drugs in an incompletely exposed population with the genetic system of *Plasmodium*.

Fig. 1 shows an outline of our model, which is deterministic. The infected human population is assumed to be partly drug treated and partly not, and the mosquitoes which bite the former and the latter are considered separately, because in mosquitoes which bite drug-treated patients there could only be self-fertilization between resistant *Plasmodia* with no chance for recombination if all the susceptibles had been killed in the blood which these mosquitoes imbibe and if there is only one form of resistance allele in the population. However, it seems probable that the mosquitoes which bite untreated patients could pick up members of several genetically different clones of *Plasmodium* so that double heterozygotes could arise and hence there would be an opportunity for recombination between resistance loci. It is known that naturally infected human beings can indeed carry several distinct clones of *P. falciparum* (see WALLIKER, 1983; GRAVES *et al.*, 1984). However, we do not know how much restriction on the likelihood of double heterozygote production there would be as a result of limitations on the number of distinct clones in one blood meal, nor do we yet know the genetic map distances between resistance loci in human malarias. Because of these two uncertainties we computed the extreme cases of no recombination and free (50%) recombination between the loci. After each round of selection and recombination, the new genotype frequencies are recycled into a new group of infected human beings and we refer to each of these cycles of infection as a 'generation'.

Comparisons are shown in Figs. 2 and 3 of the sequential use of the two drugs A and B (drug A being abandoned when 50% resistance is reached), with the use of the same two drugs as a mixture. The results are shown for the assumptions of 20% or 50%

*Present address: M.R.C. Laboratories, Box 273, Banjul, The Gambia.

Table 1—Numerical models of one round of selection by either (i) one drug, or (ii) a mixture of 2 drugs A and B. Resistance to the drugs is assumed to be controlled by two independent genes A^R and B^R. These are assumed to be randomly associated but in case (X) both are rare and in case (Y) only A^R is rare. Only 50% of the population is assumed to be drug treated and the dosage kills all genetically susceptibles and no genetically resistants. Note that the use of a drug mixture greatly reduces the rate of increase in the frequency of A^R only when B^R is rare and that the use of the mixture increases the ratio of double resistant to single resistant clones (i.e., creates linkage disequilibrium)

			Number of Clones				Frequency of:	
			A^RB^R	A^RB^S	A^SB^R	A^SB^S	Total	A^R	B^R
(X)	Initial pop., both A^R and B^R rare		10	9990	9990	9980010	10^7	0·1%	0·1%
		50% untreated	5	4995	4995	4990005	5×10^6		
	(i)	50% treated with A	5	4995	—	—			
		Total of survivors	10	9990	4995	4990005	$5{\cdot}005 \times 10^6$	0·2%	0·1%
		50% treated with A and B	5	—	—	—			
	(ii)	Total of survivors	10	4995	4995	4990005	5×10^6	0·1001%	0·1001%
(Y)	Initial pop., only A^R rare		5000	5000	4995000	4995000	10^7	0·1%	50%
		50% untreated	2500	2500	2497500	2497500	5×10^6		
	(i)	50% treated with A	2500	2500	—	—			
		Total of survivors	5000	5000	2497500	2497500	$5{\cdot}005 \times 10^6$	0·2%	50%
		50% treated with A and B	2500	—	—	—			
	(ii)	Total of survivors	5000	2500	2497500	2497500	$5{\cdot}0025 \times 10^6$	0·15%	50·02%

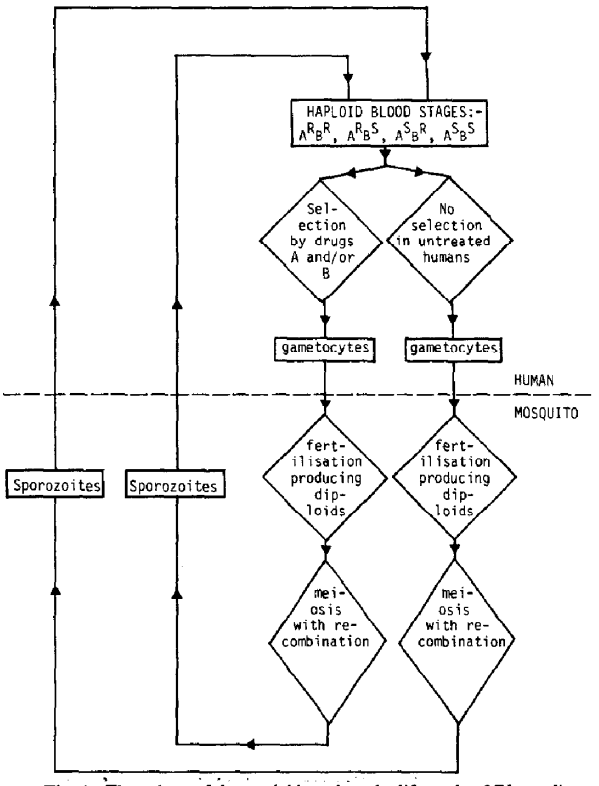

Fig. 1. Flow chart of the model based on the life-cycle of *Plasmodium* and distinguishing between parasites from treated and from untreated patients.

(Fig. 2) or 80% (Fig. 3) of the population untreated with drugs, which are assumed to kill all of the treated parasites which do not carry the appropriate resistance gene(s). Some drugs (such as pyrimethamine) inactivate gametocytes, but others (such as chloroquine and quinine) do not affect gametocytes. For the purposes of this model, gametocytes may be considered as part of the untreated fraction of the population in relation to the latter type of drug.

Figs. 2 & 3 (lower parts) indicate that if there is no recombination between the resistance genes, there is no advantage for mixed as opposed to sequential usage, in terms of the time for 50% resistance to both drugs to be reached. On the other hand, where 50% of the population is untreated and where recombination between the resistance genes is free, the mixture does considerably delay the build up of resistance (Fig. 2(i)).

Figs. 2 & 3 (upper parts) show that the relatively poor performance in the absence of recombination is associated with faster selection by the mixture for a non-random association of the two resistance genes. This is conventionally called linkage disequilibrium though, as shown in Fig. 2, it can build up even in the complete absence of genetic linkage.

Fig. 2(ii) shows that when only 20% of the population is unexposed to any drug, evolution of resistance is faster, and even if there is free recombination between the resistance genes the use of a mixture would have hardly any advantage over sequential use of the drugs.

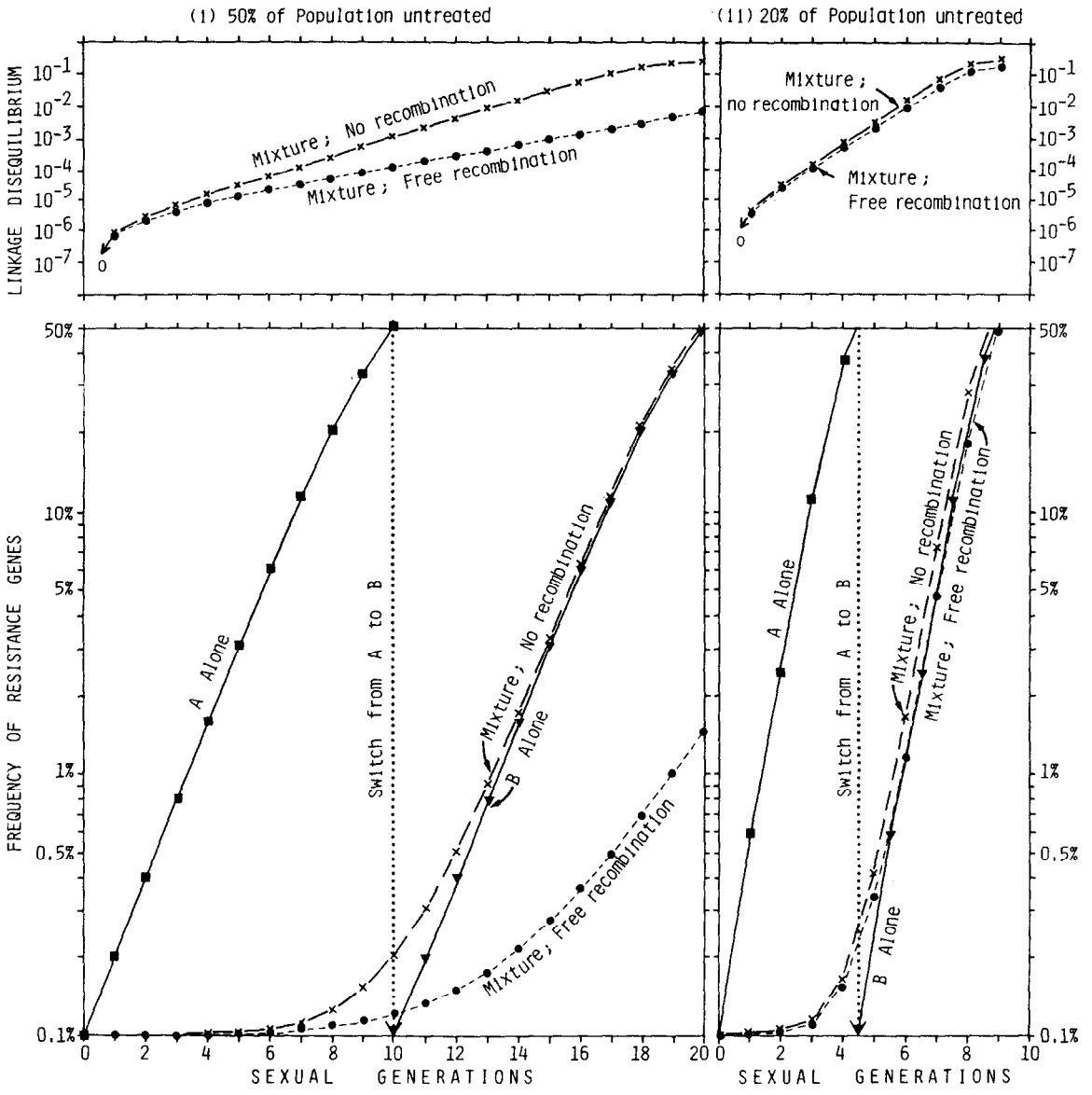

Fig. 2. Build-up in the *Plasmodium* population of the frequencies of drug resistance genes (lower graphs) and of the degree of non-random association (linkage disequilibrium) between them (upper graphs), in the cases where (i) 50% of the population are untreated with drugs, or (ii) 20% are untreated. Sequential use of drugs A and B is compared with the use of a mixture of A and B, and in the latter case the effects of no recombination or free recombination of the resistance loci are shown on both the resistance gene frequencies and the linkage disequilibrium. In the case of sequential use, the graphs show first a rise in the frequency of the gene A^R to 50% at which point a switch to drug B is made and the frequency of B^R then starts to rise. In the case of the use of mixtures, the graphs indicate the rise in A^R and B^R simultaneously. Linkage disequilibrium is defined as the frequency of the $A^R B^R$ combination minus the product of the frequencies of A^R and B^R. The initial frequency of A^R and B^R is assumed to be 0·1% and the resistance and susceptibility alleles are assumed to be neutral to selection in the absence of the relevant drug. The abscissa shows numbers of sexual generations, i.e., passages through the mosquito from one human being to the next.

Fig. 3 indicates that if 80% of the population is unexposed and if there is free recombination, the use of a drug mixture is highly advantageous. This is because under these conditions the tendency of the sexual process to halve the linkage disequilibrium at each generation more than counterbalances the relatively weak selection for linkage disequilibrium. In the case of a diploid insect the conditions under which mixtures are advantageous are complicated by the question of the degree of dominance of the resistance. However, in the case of *Plasmodium*, which is selected at the haploid stage, this complication does not arise and the outcome depends only on the initial gene frequency and the balance between genetic recombination and the intensity of selection.

From Table 1 and Figs 2 and 3 it would seem that in large natural populations the use of mixtures of non-interacting drugs would never cause resistance to

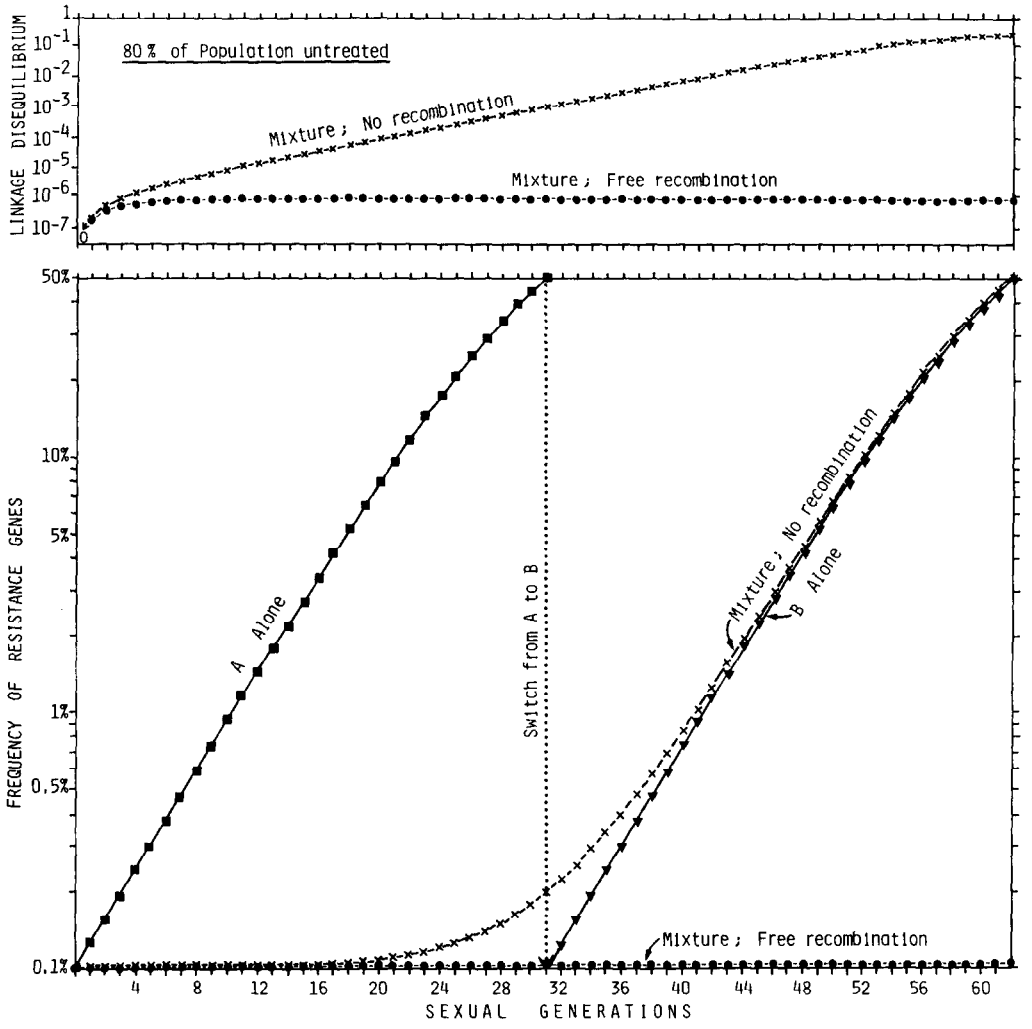

Fig. 3. As for Fig. 2 but for the case where 80% of the population are untreated with drugs.

appear faster than using the same drugs singly and mixtures could greatly delay the appearance of resistance provided that: (i) both resistances are initially rare, (ii) there is a recombination between the resistance genes concerned and (iii) many patients do not receive drug treatment.

We recognize that our models are too simple to use as a basis for strategic decisions about anti-malarial drug policy but consider that the factors of initial resistance frequency, genetic recombination and the unexposed fraction in a population should be taken into account in more sophisticated computer models, in selection experiments with animal models and in observational studies on drug-treated human populations.

Acknowledgements

We thank R. Page for assistance with programming and W. Peters and D. C. Warhurst for comments on the manuscript. The work was carried out during tenure of a bursary from the London School of Hygiene and Tropical Medicine by L.G.O.; C.F.C. acknowledges financial support from the Medical Research Council.

References

Curtis, C. F. (1985). Theoretical models of the use of insecticide mixtures for the management of resistance. *Bulletin of Entomological Research*, **75**, 259-265.

Graves, P. M., Carter, R., Keystone, J. S. & Seeley, D. C. (1984). Drug sensitivity and isozyme type in cloned lines of *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene*, **33**, 212-219.

Peters, W. (1984). Use of drug combinations. In: *Anti-Malarial Drugs*, Vol. 2. W. Peters and W. H. G. Richards (editors). Berlin & Heidelberg: Springer Verlag, pp. 499-509.

Peters, W. & Robinson, B. L. (1984). The chemotherapy of rodent malaria XXXV: Further studies on the retardation of drug resistance by the use of a triple combination of mefloquine, pyrimethamine and sulfadoxine in mice infected with *P. berghei* and '*P. berghei* N.S.' *Annals of Tropical Medicine and Parasitology*, **78**, 459-466.

Walliker, D. (1983). *The Contribution of Genetics to the Study of Parasitic Protozoa*. Chichester: John Wiley.

Accepted for publication 22nd May, 1985.