THE

EVALUATION

OF

SOME ANTIMALARIAL DRUGS IN MAN

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INTRODUCTION

Of all diseases, malaria has been considered the disease which is most widespread throughout the world and of the most overall economic importance. Some two to three million people are estimated to die directly or indirectly due to malaria each year (Findlay 1951 a). Considerably higher estimates have been made in the past, but where diagnosis becomes more precise, the morbidity figures for malaria are found to decrease and the figures for tuberculosis and virus diseases increase. Nevertheless, because of the real importance of malaria, it is natural that very considerable effort has been expended on developing and perfecting drugs to combat it. The economic changes likely to result from control are large, and expenditure on control and eradication is immense both by national and international organisations.

Much careful thought and planning goes into the early stage of development and assessment of antimalarial compounds (Davey 1946 ; Wiselogle 1946). It is perhaps surprising, in view of the large economic factors involved, how comparatively crude and uncritical have been many of the late stage assessments of antimalarial drugs, and in many countries how slow has been the acquisition of knowledge of the most effective use and application of antimalarial drugs.

Although mepacrine was first used in the early 1930's (Kikuth and Schonhofer 1935), it was not until ten years later that it was clearly shown that a prophylactic regime could be used with confidence (Fairley 1945) and that when used to cure an established infection, a high initial dose of mepacrine was definitely advantageous (Bryant 1942 ; Findlay et al. 1944). Similarily, chloroquine was first synthesised for

/antimalarial tests ...

antimalarial tests in 1934 (Findlay 1951 b), but, even twenty years later, there was considerable variation in the dosage recommendations made. As antimalarial drugs are mainly used amongst peoples with very limited resources, it is of importance for economic reasons alone to have precise information on their pharmacology and activity so that the maximum use can be obtained from the supplies available to them.

The time taken to decide upon the optimum prophylactic and curative regimes has been to some extent due to the difficulty of obtaining human volunteers to take part in controlled trials and also because of the confusion that has arisen from the number of tests that have been carried out and published of which the design and supervision have not been entirely satisfactory. These field tests have often produced conflicting results and sometimes the authors have drawn conclusions from their work which their published evidence does not necessarily justify. In those trials which have been carried out under more standardised conditions, using a mosquito colony and volunteers, there has naturally been a limitation on the number of strains which it has been possible to test, but many of the other common sources of error can be eliminated by such a specialised unit. Where the results are of major importance, controlled trials with human volunteers are undoubtedly the most practical way to obtain evidence rather than impressions.

It is useful to consider the number of sources of error or variation that may occur in conducting a trial of any drug, and particularly of an antimalarial drug. Varying results may be reported and arise from :-

(1) Failure to give the correct drug.

(2) Failure to give the correct dose of drug, or the opportunity for the subjects to obtain additional drug exists.

(3) Failure of suitable formulation of the drug.

(4) Abnormal absorption, storage, metabolism or excretion of the drug.

(5) Variation of immunity of the subjects.

(6) Variation of intensity of infection.

(7) Variation of susceptibility of plasmodial strains to the drugs.

Frequently, however, the diagnosis of malaria in the first place is wrong, and I have found this due to :-

(1) Badly taken slides (e.g. some parasites from previous patients may even be adhering in blood clot to the needle used for spreading a thick blood film).

(2) Erroneous labelling.

(3) Poor stain and technique (e.g. particles from other positive slides floating off and contaminating slides in the same staining bath.)

(4) Poor quality microscopes, lighting and microscopists.

(5) Failure in transmission of the correct report.

Variations of strain should not be immediately assumed to be the reason for failure of a drug in prophylaxis or cure without consideration and preferably evidence. It must be realised however that strains varying in their sensitivity to different drugs do exist and have been shown to exist in certain areas, e.g. quinine in the Rome area (James et al. 1932), mepacrine in Aitaipe-Wewak (Fairley 1946), proguanil in Malaya (Edeson and Field 1950), pyremethamine in India (Chakravarty, N.K. and Chaudhuri, R.N. 1953). I have been asked to investigate a number of unusual results found in subjects reputed to be taking antimalarial drugs. The first explanation usually suggested to me was that these results were due to abnormal strains. In fact, I have found that usually the diagnosis was incorrect and the fever was not due to malaria, so that, naturally, unexpected results were obtained after administering antimalarial drugs.

In a malarial endemic area, if this subject becomes of real importance, a useful method of checking the sensivity of strains is to give at first a very small dose of the drug under suspicion to some subjects showing parasitaemia. If there is rapid clearance or sudden diminution of parasitaemia with what is usually considered a very small dose of drug, it suggests that the prevalent

/strain ...

strain is sensitive to the drug and the doses normally recommended should be effective for general use. Of course, no subject who was seriously ill would be included on a test of this nature, and, after a blood slide had been taken on the 3rd or 4th day after the test dose, a full course of antimalarial treatment would be given. A test dose equivalent to 200 mgm. of proguanil for adults was used with satisfactory results on some 60 patients who came for treatment and showed parasitaemia in Nairobi. They had come from many parts of East Africa and had not acquired their infection in Nairobi. After taking a blood slide on the fourth day after dosing, they were given 600 mgm. of chloroquine base. This was done as a recrudesence of parasites would otherwise have been probable in most of the patients after such a small dose of proguanil. That clearance of parasites had occurred, temporarily at any rate, indicated that proguanil would be effective as a prophylactic against the strains involved. Whether these 60 cases constituted many strains or substrains of course is not known. Very few facts are available about the prevalence of strains or substrains of plasmodia and their possible variations.

Between the wars very valuable investigations on the chemotherapy of malaria were carried out at the Ministry of Health Malarial Laboratory at Horton Hospital, Epsom, principally by James, Sinton and Shute. During the 1939-1945 war, when malaria was causing severe dislocation amongst Commonwealth troops in the East, a large malaria research unit was set up and directed by Hamilton Fairley at Cairns, Australia. There, by carefully designed experiments and with the aid of volunteers, numerous facts were rapidly obtained of practical importance in the control and treatment of malaria. Within a year, more was learned about the pharmacology and activity of mepacrine in man than had been learned in the previous ten years. In the same way, similar large scale but less fundamental trials were carried out in the U.S.A. in the latter war years. There, Coatney and Alving have been able to continue work

/in penitentiaries ...

in penitentaries mainly using P. vivax. But with the advent of peace, facilities for trials with volunteers diminished in most countries. Much of the mystery of malaria had by then been explained, and for those who could afford them, adequate and reliable drugs were available. Nevertheless, those responsible for the care of the people in poor communities were not yet able to bring the advantages of freedom from malaria to them all. If malaria eradication is to be undertaken rapidly, still cheaper drugs are required, and an even more precise knowledge of the correct dosage, so that overdosage and waste can be reduced. Recent reports, as yet unpublished, from W.H.O. conferences in West and East Africa suggest that insecticides alone, except in special sites, are rarely effective, and a greater use of chemotherapy combined with insecticides is desirable to achieve malaria eradication.

Imperial Chemical (Pharmaceuticals) Ltd. wished to examine certain actions of proguanil. They also wished to investigate other compounds which Davey (Personal Communication) had found were showing important antimalarial activity against P. gallinaceum and P. knowlesi. A decision had to be reached within a reasonably short time as to what the place of these compounds might be amongst established antimalarial drugs. In addition, there were threats of war in the East and reliable reports from Malaya of resistance to proguanil in treatment, the importance and scale of which was then unknown. No existing facilities were available within the Commonwealth to collect this information, so Imperial Chemical (Pharmaceuticals) Ltd. set up a unit of its own in Nairobi with the approval of the Colonial Medical Research Committee, the Director of Medical Services and members of the Kenya Government.

The work described in the following pages was carried out at this unit in Nairobi between 1951 and 1955 by myself, but it would not have been possible without

/the cooperation ...

the cooperation of many people - Dr. Farnworth Anderson, Director of Medical Services, Kenya; Dr. A.T.G. Thomas, M.O.H., Nairobi; Dr. E.J. Foley, Dr. R.B.Heisch of the Kenya Government; Dr. J. Field, Dr. T. Wilson and Dr. J.F.B. Edeson of the Medical Research Institute, Malaya; the War Office, and particularly the staff of the East Africa Command Royal Army Medical Corps. I received assistance on different experiments from numerous colleagues to whom I am greatly indebted, especially Dr. J.H.D. Roberts, Dr. J.W. McAllan, Dr. R.J. Murray, Mr. H. Moores, Mr. G.R.C. Van Someren, Mr. H. Jones, Mr. L. Powell, Mrs. J. Bodington, Mrs. J. Young and Mrs. E.S. Duncan. From time to time also I received much valuable help, encouragement and suggestions from my colleague Dr. D.G. Davey who visited the unit on three occasions, as well as Sir Neil Hamilton Fairley, Sir Gordon Covell and Mr. P.G. Shute, to all of whom I am most grateful. To all the volunteers for their willing cooperation a very special debt of gratitude is due and an obligation to ensure that the knowledge acquired at the expense of their discomfort is utilised for the fullest benefit of others.

SECTION ONE

A DESCRIPTION OF METHODS

In 1951, work was started at the Imperial Chemical (Pharmaceuticals) Ltd. laboratory in Nairobi on testing antimalarial drugs, and, as will be explained in this section, both non-immune Europeans and Africans likely to have relatively little immunity acted as volunteers. To have used only non-immune volunteers would, of course, have been ideal for our purposes, but only some 70 men were available. However, we were fortunate in having some 300 Africans likely to have relatively little immunity who were ready to co-operate on these tests. As is explained, for certain types of tests we consider volunteers who may have some degree of immunity are quite suitable. It must also be remembered that probably 98% of all antimalarial drugs are used by people who may already have some varying degree of immunity.

Obtaining Infected Mosquitoes :

All the mosquitoes used were from our own laboratory colony of Anopheles gambiae raised by the method of Moores (1953). They were infected from patients for whom fever therapy has been prescribed and in whom the parasite was carried and passaged by blood or mosquito infection. Great difficulties were encountered in obtaining large numbers of infected mosquitoes due to the variability of infectiousness of different gametocyte 'carriers'. It was not always possible to obtain a sufficient number of gametocytes in a 'carrier', and further, mosquitoes kept under identical conditions and feeding on different 'carriers' with apparently similar gametocyte counts of the same strain would sometimes produce very different infectivity rates. Our experience led us rarely to feed mosquitoes if the gametocyte counts were less than 100 per c.mm., but even so, we quite

/frequently failed ...

frequently failed to infect a single mosquito in a batch of several hundred mosquitoes. If the gametocyte count was less than this, the infection rate of the batches, if any infection was obtained, was very low indeed. From the many batches which we dissected, we found nothing to suggest that subjects with low gametocyte counts ever infected a high proportion of mosquitoes (see also Muirhead Thomson 1954).

During some five years' work, we have attempted to obtain infection from about 90 gametocyte carriers. In spite of this experience, we can lay down no absolute rules as to how a good infection of mosquitoes is most likely to be obtained. We could not link our results with the age of the gametocytes, or whether trophozites were present or absent, or whether males exceeded females, or vice versa. On occasions, a gametocyte carrier would fail at all stages to infect any mosquitoes, though other mosquitoes kept under identical conditions but feeding on a different gametocyte carrier, would readily become infected. Our highest infection rate was obtained in a batch which was exposed to one gametocyte carrier on Day 1 and 2, and a second carrier on Day 3 and 4. Both carriers were infected with a Malayan strain. On subsequent dissection, all 66 surviving mosquitoes in that cage were found to be heavily infected with sporozoites.

Normally, the salivary glands of the mosquitoes used were flooded with sporozoites, and unless sporozoites were seen in numbers and quite readily, the mosquitoes were classified as non-infected. The batches of mosquitoes which we used to bite volunteers had shown sporozoites in the salivary glands for at least two days and not more than sixteen days. (Under our conditions, sporozoites usually appeared in the salivary glands on the 11th or 12th day after feeding). It has been suggested that younger or older sporozoites than these may not cause infection. We had some opportunities to examine this, and certainly obtained infection from a mosquito whose

/sporozoites ...

sporozoites were unlikely to have been in the salivary glands for more than one day and also from a mosquito whose sporozoites were likely to have been in the salivary glands for at least 26 days, but to be safe we kept within the times mentioned.

Two abnormalities of sporozoites were encountered, and when they were present, the affected batches were not used for chemotherapeutic trials. On two occasions, for a period of about a month, we noted that a very high proportion of the sporozoites were degenerate; they were short, shrivelled up and curved in appearance. The cause of this was unknown. On three batches from the same strain, in different years, we noted the sporozoites appeared to be sticking together in clumps or rafts. Sometimes these 'sticky' sporozoites were slightly narrower and showed the nucleus more prominently than usual. We were unable to account for this phenomenon. We found that infection could be transmitted by mosquitoes in which the sporozoites appeared to be 'sticky', but we did not use them in critical tests.

Initial dissections were made of the salivary glands of a small proportion of each batch of mosquitoes that had fed on a gametocyte carrier at least twelve days earlier, to find out if normal sporozoites were present and were likely to be present in a high percentage of the batch.

Infecting the Volunteers :

To carry out the experiments, a few mosquitoes from an infected batch were placed in a small spherical lamp glass, diameter of about two inches, with mosquito net covering both ends. The glass was then held by the volunteer on his arm or leg and the mosquitoes allowed to feed for about half-an-hour through the mosquito net cover. Within a few minutes, most of the mosquitoes would feed readily, even in the daylight, provided that they had had no blood feed within 48 hours. They tended to probe the

/skin ...

skin considerably, and since sporozoites along with the saliva and anticoagulant are probably injected during the probing, the challenge we gave to the drugs can be regarded as severe. Often, three mosquitoes would leave some ten or twelve wheals. In natural conditions in a holo-endemic area, it is rare to encounter more than 5% of female anopheles showing any sporpzoites at all. Depending on the infectivity of the batch, we varied the number of mosquitoes in each pot to ensure that whatever the infectivity of the batch used, two or three were likely to be infected mosquitoes. As mentioned later, for certain experiments, an even greater number of infected mosquitoes were used.

After biting, a proportion of the mosquitoes containing blood were dissected and their infectivity checked to supplement the details of that batch. Throughout these tests, also as a control, a sample of mosquitoes would be allowed to bite a volunteer not protected by drug treatment; usually, this was incorporated in the passage of the strain or as part of the test of a volunteer's susceptibility to infection.

In our initial test we were able to use batches of mosquitoes which were about 85-90% infected. On some of the later tests we had to include in the batches used somewhere the infectivity rate was as low as 33%.

The Volunteers :

The African volunteers were chosen from tribes normally resident in areas where malaria is endemic in small degree only, if at all, so that, taken as a group, they were as little immune to malaria as adult Africans born and raised in Africa are likely to be. The tribes from which they came have an extremely low incidence of sickle cell trait. Most of these volunteers were tested, and none was found to possess the trait. The European volunteers were all British soldiers who had arrived recently in Kenya, having previously lived in non-malarious territories, and were carrying out normal duties at a

/headquarters ...

headquarters or hospital unit in Nairobi. There was, so far as could be found out, no malaria transmission occurring in Nairobi at the time of these experiments, as the malaria control measures there are of a high standard.

The volunteers were seen daily and thick blood smears were examined and temperatures taken at least once daily by myself or European technicians. If any man had any complaints whatsoever, additional examinations were made. Particular care was taken to see that the entire dose of the drug under examination was swallowed.

The Infectivity of P. falciparum from One Area for Mosquitoes from Another :

It is worth recording that we found that our Anopheles gambiae, originating from the Nyanza Province of Kenya, was able to transmit the Malayan strains of P. falciparum with no more difficulty than African strains of P. falciparum. James, Nicol and Shute (1932) found that their colony of an English strain of A. maculopennis could transmit strains of P. falciparum from the South of Europe but not an Indian strain of P. falciparum. Later, Shute also failed with that strain of mosquito to transmit three African strains of P. falciparum. Shute & Maryon (1951), at the Ministry of Health Malaria Laboratory, Epsom, showed that A. stephensi from Delhi was an efficient carrier of a West African strain of P. falciparum, whilst an English strain of A. atroparvus maintained there was a poor carrier.

The Strains of P. falciparum Used :

Throughout these tests, in which we used artificial infections, we worked entirely with P. falciparum. We did so because this species is the most widely spread species, the most dangerous and, therefore, of the greatest overall importance. The early reports of the failures of proguanil which first prompted this work involved only P. falciparum, and it is generally true that reported failures in prophylaxis with most drugs concern only P. falciparum. P. vivax can be a nuisance when people

/return ...

return from malarious areas and stop their prophylaxis, but it rarely matters in the problem of prophylactic "breakthrough". It is not without interest that larger doses of drugs are required to suppress the erythrocytic forms of most strains of P. falciparum than are required to suppress most strains of P. vivax.

In the experiments as a whole, use was made of five "strains" of P. falciparum. Details of these strains are as follows :-

(i) The Mombasa Strain :

This was our original strain and was maintained for five years by mosquito and blood transmission. It was sent to us in Nairobi from Mombasa in response to our request for blood containing a strain of P. falciparum found in any patient who was likely to have acquired his malaria infection in that area. It was transmitted readily by Anopheles gambiae, and we found that it caused a virulent infection in patients requiring pyrotherapy, a fact which was confirmed by Garnham in neurosyphilitic patients in Europe. (Personal communication).

(ii) The Lake Victoria Strain :

This was taken from a patient who was likely to have acquired his infection on the northern shore of Lake Victoria.

(iii) and (iv) The Malayan 1 and 2 Strains :

These were sent to us at different times in heparinised blood from the Tampin area in Malaya with the kind cooperation of Drs. Field, Wilson and Edeson of the Medical Research Institute, Malaya. A request was made for parasites found to be resistant to proguanil in the erythrocytic stage, i.e. the infected blood was taken from patients in whom treatment of a clinical attack of malaria with proguanil was unsuccessful. We wished to gauge the degree of resistance in the pre-erythrocytic stage, to investigate some aspects of the resistance in the mosquitp cycles, and the cross resistance between drugs. Malayan No. 1 was also highly resistant to pyremethamine (Robertson, Davey & Fairley 1952), but Malayan No. 2 did not show this cross resistance to the same degree.

(v) The Makueni Strain :

This was investigated at the request of Dr. S. Avery Jones. He had given a mass treatment of pyremethamine, using 100mg. as a single dose for adults and with proportionate reduction for children, to all inhabitants of an isolated village 100 miles from Nairobi (Avery Jones 1954). One week later, the eighth day, the blood slides of 100 of the school children examined were free of parasites, except for one child. Prior to the dosing, 45% had shown asexual parasites. This seven-year old child was given doses of 50 mg. of pyremethamine on the eighthand again on the ninth day after the initial dose, but on the fifteenth day asexual parasites were still numerous. Avery Jones considered it important to find out whether this one child was showing an abnormal metabolism of the drug, or a refractory strain was affecting this one child only in this isolated community where he had lived amongst the others for several years. Pyremethamine had never been used there, and it was thought to be very unlikely that the child had had even a single tablet of proguanil, as proguanil had never been supplied to that village. The strain was subinoculated from the child on the sixteenth day into a patient requiring pyrotherapy and mosquitoes were later infected from that patient. Like all the strains we have used, the virulence of the strain appeared quite normal and patients required to have their fever controlled by small doses of quinine or chloroquine.

The Infectivity of Different Strains of P. falciparum in the Volunteers :

It is interesting that whether African patients of similar origin were infected with an African strain or a Malayan strain, there was no obvious difference in the symptoms or course of the malaria, or in the number of those who required interference with small doses of chloroquine or quinine to control the infection. There were, of course, great variations in the course of the infection, depending on the origin of the patient and the degree of endemicity of malaria in his tribal area.

As an example, one neurosyphilitic patient who had been in hospital for over ten years in Nairobi, where malaria transmission was virtually not occurring, was infected with Malayan No. 1 Strain. His thick blood smears showed a few trophozoites persisting for ten days; he had no complaints whatsoever, and no pyrexia was noted at any time. This man came originally from a holoendemic area and did not have sickle cell trait. Similar, but slightly less marked, examples all suggest that if there has been intensive infection throughout childhood, a very definite and lasting immunity is built up to a strain of P. falciparum from several hundred miles away, or even from another Continent. There are many unexplained reports of people moving to a new area and soon developing an acute malaria infection in which a change of temperature or height is blamed for allowing a recrudescence to occur. There are also reports that patients living in one malarious valley move over the hill to another valley and there acquire a new infection to which they are reported as having virtually no immunity. If these observations are correct, it is difficult to reconcile them with ours. Thus, in our experiments the different response to infection appeared to be much more dependent on the origin and so on the quantitative degree of acquired immunity of the patient than on the origin of the strains that were used. Reinfection with a homologous strain, however, has been made to show that a clear cut specificity of immunity can be produced for all human species of plasmodia (Boyd 1949).

Types of Causal Prophylactic Experiments :

Most of the experiments fell into two types :-

(1) Multiple session biting type. In this type, a group of volunteers, usually four to ten in number, were exposed to infected bites on many occasions over a period of some weeks whilst taking the compound being tested. The intensity of the infection was arranged to be greater than was ever likely to be encountered under field conditions.

Usually, an average of at least one or two

/infected mosquitoes ...

infected mosquitoes bit each volunteer daily. With increased education, with the knowledge that malaria is transmitted by mosquitoes and with the greater use of residual insecticides, the proportion of people exposed to intensive infection is certainly becoming less. In and around many of the larger towns in tropical countries, both community and personal measures are taken which have reduced the opportunity for contact between man and mosquito. Areas and villages where populations are liable to be exposed to a heavier infection than we induced are diminishing rapidly throughout the world.

(2) Single session biting type. In this type of experiment, the volunteers were exposed to infection on one occasion only and the drug was given once only at a definite time in relation to the time of biting. To make this one challenge severe, we increased the number of mosquitoes used so that an average of 3-6 heavily infected mosquitoes bit each volunteer. At first, in this type of experiment, three volunteers were grouped together and each given the same dose that required investigation. As our experience of the immunity likely to be encountered in the volunteers on these single biting tests grew, we usually made up a group of one man who was likely to be very susceptible, and another who, from his origin, we might have some doubt as to his degree of immunity but who was anxious to volunteer for the test. Almost always they both showed the same outcome; malaria did develop in both or did not develop in either. Where doubt existed and the result was important, more volunteers were included. It was always kept in mind that with a certain dose of drug, failure of protection was a significant result however few the failures. Complete protection, on the other hand, unless the number of volunteers was considerable, required confirmation.

The drugs we were using on this type of single biting session tests were of the causal prophylactic type and all are related to proguanil. The early exo-erythrocytic forms of P. falciparum are much more sensitive to the drugs

/of this type ...

TABLE I

The action of proguanil against P.falciparum in single session biting tests.

Strain and volunteers.	Number of hours dose given after exposure to infection.	Dose of proguanil.	Number of volunteers.	Results	
				Complete protection.	Overt malaria
New Guinea strain and non-immune European volunteers*	+ 24 hours	100 mgm.	1	0	1
	+ 48	100	3	3	0
		50	2	2	0
		25	2	1	1
		10	2	1	1
	+ 130	100	2	2	0
		50	2	2	0
		25	4	4	0
		10	2	2	0
	+ 168	300	1	0	1
Mombasa strain and African volunteers.	+ 0	100	2	0	2
	+ 24	100	1	0	1
	+ 48	100	5	5	0
		75	6	5 **	1
		50	3	1	2
		25	2	0	2
Lake Victoria strain and African volunteers.	+ 48	75	2	2	0
		50	1	0	1
		25	1	0	1
Makueni strain and African volunteers⁂	0 hours and daily for four subsequent days.	100	2	2	0
	+ 48	200	3	0	3
Malayan I strain and African volunteers⁂	+ 48	300	2	0	2
Malayan II strain.⁂	+ 48	300	2	0	2

* Extracted from Fairley 1946.
** Includes one non-immune European volunteer.
⁂ "Resistant" strains - see text.

of this type so far investigated than the erythrocytic forms. It may be that when the sporozoite is first introduced into man, its metabolism is slow or insusceptible to the drugs, but that it becomes increasingly susceptible at the later part of the early exo-erythrocytic phase when its metabolism may alter. Thus, a small dose of causal prophylactic drug given on Day 5 of the early exo-erythrocytic phase, the fifth after the mosquito bite, may be effective. If given on Day 2, however, a much larger dose is required. This may be because the parasite requires a greater concentration to kill it then, Day 2, or merely that by giving a large dose there will still be sufficient drug persisting and available to kill the parasite at a later more sensitive stage, such as on Day 5. This was well shown by Fairley (1946) in non-immune volunteers who were given proguanil at certain times after being bitten, and we showed it to be true not only of proguanil (Table I), but of Compound 5943, which may be described as proguanil with an additional Cl. group in position 3 of the benzene ring, of 10580, a metabolite of proguanil, and of 10732, which is a metabolite of 5943. (See Table I).

It can be seen, Table I, that 100 mgm. of proguanil, when given 24 hours after the volunteers had been bitten by mosquitoes, was unable to prevent malaria developing, but when given 48 hours after being bitten, not only did 100 mgm. prevent overt malaria developing, but doses as low as 25 or 10 mgm. were also partly effective. If, however, 300 mgm. was given 168 hours after these non-immune volunteers had been bitten, malaria did develop. This result , at 168 hours after exposure, was because some of the parasites had passed from the early exo-erythrocytic phase, when they are very sensitive to proguanil, to the erythrocytic phase, when they are less sensitive.

The fact that exo-erythrocytic forms of P. falciparum are most susceptible to drugs of the proguanil type 2 days or more after the entrance of sporozoites and

/least susceptible ...

least susceptible in their very early life has an important bearing on the potential use of the drugs for once-weekly prophylaxis. It means that a dose to be given once-weekly must be effective, even when it is given at the time mosquitoes bite, because, if it fails, parasites can be expected to be released into the blood about 6 days later, i.e. before the next dose is due. It means, too, that the single biting test can be used to obtain very quickly knowledge of whether drugs of the proguanil type will, or will not, be effective for weekly prophylaxis, and the size of the dose necessary. We used this method considerably in our work.

The Bearing of Immunity on Causal Prophylactic Experiments :

It is usually considered that immunity is a humoral mechanism exerted against the erythrocytic forms (see the review by Covell, Coatney, Field and Singh 1955). It can be shown that, when a non-immune subject and a subject with a high degree of immunity are infected, the early exo-erythrocytic forms develop normally in each and parasites pass normally from the tissue to the blood phase. Once in the blood phase, the parasites in a non-immune subject multiply rapidly, especially if the infection has been heavy, and overt malaria develops between the eighth and fourteenth day. A non-immune subject, if untreated or given minimal treatment, would show parasitaemia of varying intensity for many months. Any immunity the subject may possess comes into play to suppress these erythrocytic forms. If the immunity is already exceedingly highly developed, the blood forms of the parasites are suppressed so effectively that, though present in small numbers, they do not even become visible in a thick blood film. Frequently, however, intermediate stages of immunity are found in which parasites become visible in a thick blood smear, perhaps after a delayed incubation period and in limited numbers, and parasites can be found there for only a few weeks. In general, the greater the immunity the less frequent are gametocytes found, though gametocytes are never found during the first

/fifteen days ...

fifteen days after a mosquito infection with P. falciparum. Where immunity is high, there may be little or no temperature or symptons and the immunity mechanism is sufficient to overcome and almost suppress the visible parasitaemia without the administration of any schizonticidal drug. This was shown by us amongst men of the Jaluo tribe who were infected in the course of another experiment designed and described by Allison (1954). This tribe live in a holoendemic area and were not, of course, accepted as volunteers for any tests connected with chemotherapy as they were likely to have a high degree of immunity. In these tests it was found amongst those without sickle cell trait when exposed to mosquito and blood infection from a local and a foreign strain (Malayan No. 2) that parasitaemia did actually occur, though it was light and transient in fourteen out of fifteen volunteers. In the other group of this highly immune tribe, who all possessed sickle cell trait, parasites never became visible in thick films in thirteen out of fifteen men, so that immunity plus sickle cell trait appeared to have a very considerable suppressive action on visible parasitaemia.

It is well known that the dose of anti-malarial drugs of the purely schizonticidal type, such as quinine, mepacrine, chloroquine, amodiaquine, required to suppress malaria in a non-immune is larger that that required by a semi-immune. This must be because the non-immune does not possess any immunity of his own to have an additive effect on the suppressive action of the drug.

With drugs which, as well as having some schizonticidal activity, have a marked action on the early exo-erythrocytic forms, such as proguanil, pyremethamine, 5943, 10580, 10732, the problem is rather different. As we have explained, whether the subject has immunity or not seems to make little or no difference to the course of the early exo-erythrocytic forms. If the dose of the causal prophylactic drug is ineffective, parasites will be able to pass into the blood. We were interested in all or none effects. Whether the parasites appear in the

/blood smears ...

blood smears rapidly and in large numbers within the normal incubation period, as in the non-immune, or only in small numbers and perhaps later than the normal incubation period, as in the semi-immune, is of no account. What is important is that their appearance shows that parasites have not been eradicated in the early exo-erythrocytic stage by the particular dose given. To measure the required dose of a causal prophylactic drug, therefore, we consider it is of little importance whether the volunteers possess a moderate degree of immunity or are non-immunes. Obviously, however, volunteers such as the highly immune Jaluos, with sickle cell trait, who have been mentioned above, would be unsuitable for these tests. As soon as the first definite parasites were seen in the blood of these volunteers, we gave a full curative treatment so that symptoms were reduced to a minimum. The symptoms sometimes passed practically unnoticed if we had been particularly diligent in our search and found undoubted parasites at a very early stage after they had passed from the early exo-erythrocytic forms into the blood.

Comparison of Indigenous Africans and Non-immune Europeans and their Required Causal Prophylactic Dose :

The results Fairley obtained in single session biting tests, using the causal prophylactic drug, proguanil, a New Guinea strain of P. falciparum and non-immune volunteers gave results very similar to those we obtained, using proguanil, two East African strains of P. falciparum and indigenous African volunteers. (See Table I). For example, when the drug was given 48 hours after exposure to infection, a dose of 50 mgm. of proguanil was just able to protect non-immunes against the New Guinea strain. When the Mombasa or Lake Victoria strain was used, that dose failed to protect 3 out of 4 indigenous Africans, but 75 mgm. protected 6 out of 7 indigenous Africans and one non-immune.

Similarly, as is demonstrated in a later section on 5943, doses of 15 mgm. of 5943, given at the time of exposure, were able to protect not only numerous Africans

/exposed ...

exposed to the Mombasa strain, but two non-immune Europeans; malaria, however, did develop in one indigenous African who had taken 10 mgm. of 5943 at the time of exposure to this strain.

As our experience and knowledge of the response and susceptibility of volunteers increased, we decided that we could obtain valuable information by allowing a group of two or three volanteers to be exposed to infected mosquitoes on one occasion only, and dosing them with the compound under investigation at a known time in relation to that exposure. This was simpler than carrying out the multiple session type of biting experiment. Using the single session biting test, we found we could obtain a relatively consistent measure of the minimum causal prophylactic dose required for these volunteers when dosed and exposed to infection at specific times. We could thus obtain information about the rate of disappearance, due either to excretion or destruction, in man of these compounds, some of which cannot be estimated chemically in the blood. We found that if we followed Fairley's example and established a yardstick, we, or other workers, could then accurately measure and compare the response of other strains and other drugs.

For testing schizonticides, of course, this technique is of little value, because immunity may take a large share in causing the suppression of the malaria parasites. There are then two factors involved, immunity and drug activity, and one of them, the immunity factor, is variable and virtually immeasurable. Therefore it is impossible to assess accurately by tests with semi-immunes the suppressive dose required for non-immunes or semi-immunes with their varying degrees of immunity.

SUMMARY

The methods used in the evaluation of a number of anti-malarial drugs in man are described and discussed, including :-

1. The difficulty of obtaining suitable gametocyte carriers of *P. falciparum*.

2. The origin of the five strains examined.

3. The carriage of a foreign (Malayan) strain of *P. falciparum* by African *Anopheles gambiae*.

4. The immunity of the volunteers.

5. The suitability of African volunteers with partial immunity for causal prophylactic tests.

6. The advantages of the single session biting test over the multiple session biting test for examining causal prophylactic drugs.

SECTION TWO

THE PROTECTIVE EFFECT OF PROGUANIL AGAINST AN EAST AFRICAN STRAIN OF *Plasmodium falciparum* TRANSMITTED BY *Anopheles gambiae*

Shortly after proguanil was introduced into East Africa (late 1946 and early 1947) criticisms were voiced that it was not always effective in prophylaxis. Much of the criticism would be best described as hearsay, and it was virtually impossible to obtain precise facts. The majority of people appeared satisfied with the drug, but whether the minority who were dissatisfied were careless with their medication or were misdiagnosed - "fever" is all too frequently synonymous with malaria in Africa - could not be decided. Controlled experiments in volunteers were clearly called for, so arrangements were made accordingly.

The purpose of the experiments was to investigate the protection afforded by proguanil to subjects regularly and frequently exposed to the bite of mosquitoes known to be infected with *P. falciparum*. The subjects were African volunteers chosen from tribes normally resident in areas where malaria is endemic in small degree only, if at all. In other words, they were as little immune to malaria as adult Africans born and raised in Africa are likely to be. Blood smears were taken from prospective volunteers daily for several weeks before a final selection was made, and at the end of the experiment, in the event of apparent complete protection being conferred by the drug, susceptibility tests were made on those who had participated in the experiments.

Most criticism of proguanil in East Africa emanated from the coastal area, and the reported failures were concerned entirely with *P. falciparum*. For these

/experiments ...

experiments, therefore, a strain of this parasite was sent to us from a patient who had contracted malaria in the vicinity of Mombasa. Blood taken from this person was inoculated into patients at a mental hospital who required fever therapy and the parasite has since been serially passaged in them. Those showing a suitable number of gametocytes were used to infect Anopheles gambiae. This mosquito is the most important vector of malaria in Africa, but it had not previously been bred in large numbers in the laboratory and much time had to be given in the beginning of the work to the establishment of a successful colony. The methods that led to success have been described by Moores (1953).

As mentioned earlier, one of the most tantalizing features of the work was the procurement of suitable gametocyte carriers. Not more than about 20 per cent. of the patients inoculated exhibited what we thought would be a sufficient number of gametocytes to infect heavily a high proportion of the mosquitoes that fed. If we used subjects showing more than about 100 gametocytes per c.mm., and allowed the mosquitoes to feed on four or more successive days, we obtained sometimes an infection rate in the mosquitoes varying between 90 and 100 per cent. Sometimes, however, patients with very much larger numbers of gametocytes have occasionally failed to infect mosquitoes.

The Plan of the Experiments :

The prophylactic treatments chosen for the protection of the volunteers are in common use in Africa and other parts of the world, but emphasis (in the sense that more volunteers were used on them) was given to those which might be used for the protection of Africans themselves. Thirty-five volunteers were arranged in groups as follows :-

Group 1 comprised five volunteers who received one tablet of 0.1 gramme daily.

Group 2 comprised 10 volunteers who received a tablet of 0.1 gramme at 3- and 4-day intervals (they were given one tablet on the Thursday and one on the Sunday of each week).

Group 3 comprised 10 volunteers who received 0.3 gramme once weekly (on Wednesdays) and were bitten every day if possible including the day on which proguanil was given.

Group 4 comprised 10 volunteers who received 0.3 gramme once weekly and were bitten every day if possible except on the day on which proguanil was given.

The distinction between groups 3 and 4 was made because of the evidence first presented by Fairley (1946) that the early or primary exo-erythrocytic forms are least susceptible to proguanil during the first 24 hours following the injection of sporozoites. If the results obtained in Group 3 had been less satisfactory than those obtained in Group 4 this might have been the explanation, and it would have had an important bearing on the use of proguanil in the field.

The procedure adopted with the four groups, except for the difference in drug treatment, was the same. Exposure of the volunteers to the bites of infected mosquitoes started the day after the first treatment dose was given, and was continued every day if possible for 2 months. At each biting session, sometimes one, but usually two or three and, occasionally, as many as five or six, mosquitoes were fed on a volunteer. The number of infected mosquitoes available was not always sufficient to make daily exposure the rule, but rarely did any volunteer go longer than 2 days without being bitten. During the 2 months of biting, each volunteer was bitten by about 100 mosquitoes. Actually, under the conditions of the experiment, the mosquitoes tended to probe considerably, as demonstrated by the number of subsequent wheals, before really feeding, and since many sporozoites are probably injected during probing, the challenge to the drug can be regarded as a severe one, far more severe than would occur in most malarious areas.

The infectivity of each batch of mosquitoes used in the experiment was checked by dissection and by allowing a sample of two or three to bite a volunteer, not protected by drug treatment, during each of the 8 weeks of exposure. Seven of these eight volunteers developed parasitaemia. The eighth, who failed to develop parasitaemia within 3 weeks, was later proved to have immunity. In addition, dissections were made, sooner or later, of the greater proportion of mosquitoes used in the experiments, and the point checked that sporozoites were present in the salivary glands. With one exception, all the batches used were between 95 and 100 per cent. infected; about two-thirds of the exceptional batch were infected but, at the time it was used, three to six mosquitoes were fed on each volunteer to overcome the defect.

Treatment commenced the day before the first exposure to infected mosquitoes, continued throughout the biting period, and for one week afterwards. There then followed an observation period of one month, at the end of which, if nothing had happened, each volunteer was given his susceptibility test. This test was made by allowing one or two mosquitoes to feed on each volunteer on two successive days. The susceptibility test, therefore, did not offer anything more stringent than the volunteers had already suffered during the period that the drug was being administered. Throughout the experiment, thick blood films were examined daily, and morning temperatures taken, except during the susceptibility test when twice daily blood films and temperatures were taken.

The experiment was essentially one to test the causal prophylactic action of proguanil, i.e. its action against early exo-erythrocytic forms, because it is highly unlikely that, if the action were only suppressive, the short after-treatments of two doses of 0.1 gramme (Group 2) or one single dose of 300 mgm. (Groups 3 and 4) would have achieved radical cure.

Results :

Group 1. At one time during the experiment the supply of infected mosquitoes appeared to be in danger, so the attempts to infect the five volunteers receiving 0.1 gramme daily were discontinued after an exposure period lasting 46 days. This group was chosen because results with the three remaining groups, in which treatment was less adequate, were excellent.

None of the volunteers in this group ever gave any evidence of becoming infected and all proved susceptible when tested, parasites being found in all of them within 11 to 16 days of first being bitten.

Group 2. The 56 days of biting and the month's observation period passed uneventfully with this group except in the instance of one volunteer. This man showed parasites in his blood on the 15th day after first being bitten and the next day, with still more parasites in his blood, his temperature was 101.5^{0}. He was then treated immediately. We were at a loss to explain this case and suspect that somehow or other he evaded taking his proguanil. The incubation period of 15 days falls within normal limits, and it does not suggest that any drug impeded the progress of the disease. We tested him again 3 months later. He was given the original treatment (0.1 gramme twice weekly) and over a period of 5 weeks he was bitten every day except on 2 days. Altogether, 89 infected mosquitoes fed on him. The second experiment proceeded uneventfully, and at its termination he was shown to be still susceptible. The success of this second experiment with him leads us to believe that evasion of treatment was responsible for the failure of the first. Subsequent enquiries and observation pointed to his being of a psychopathic personality.

The remaining nine volunteers in this group never exhibited parasites or symptoms during the experimental period and they were therefore subjected to susceptibility tests. Seven of them gave good responses,

parasites being found within 10 to 15 days of the first biting. The other two call for comment. Volunteer No. 38 was first bitten by four mosquitoes, two on each of two successive days, all four of which were shown to be infected by subsequent dissection. No response was elicited during the next 37 days, and he was then bitten again by two mosquitoes, one on each of two successive days. Again there was no response during the next 23 days, and he was finally bitten by three mosquitoes on one day. He showed a few parasites 16 and 18 days after this third biting and thereafter appeared negative. We regard him as having a very considerable degree of immunity.

Volunteer No. 9 was bitten on three occasions. On the first he was bitten by three mosquitoes; 24 days later by two, and again after 24 days by four. The mosquitoes used were shown to harbour sporozoites of suitable age, but the volunteer never showed parasites or symptoms. We regard him as immune.

Group 3. None of the 10 volunteers in this group exhibited parasites or symptoms throughout the exposure and observation periods, and they were therefore subjected to susceptibility tests. Eight of them responded satisfactorily, parasites being found in them within 9 to 20 days of being bitten. The results in the other two need comment. Volunteer No. 24 was first bitten by four mosquitoes (sporozoites were afterwards found in them), two on each of two successive days, and scanty numbers of parasites appeared on the 15th and 16th days after the first biting, but on these two days only. He was bitten again a month later with one mosquito on one day and two on the next, and again only scanty numbers of parasites were found on the 15th and 16th days. He was then treated. We regard this volunteer as having very considerable immunity.

Volunteer No. 59 was bitten by four mosquitoes (proved positive), two on each of two successive days, and showed a few parasites on one day only, 33 days after

/his first bite ...

TABLE II

Summary of results.

Group.	Treatment.	Exposure period	Number of volunteers	Failures of proguanil to give complete protection	Susceptibility tests		
					Good	Poor	Number not tested
1	0.1 gramme daily	46 days	5	0	5	0	0
2	0.1 gramme twice weekly	56 days	10	1?	7	2	0
3	0.3 gramme once weekly	56 days	10	0	8	2	0
4	0.3 gramme once weekly (volunteers not bitten on proguanil day	56 days	10	1?	7	0	2

his first bite. He was bitten again, 31 days after this, by four infected mosquitoes all feeding on the one occasion, and again he showed only a very few parasites 14 days later. We regard this volunteer as also having very considerable immunity.

Group 4. Nine of the 10 volunteers in this group also remained free of parasites and symptoms throughout the period of exposure and observation. The exception developed malaria on the 12th day of the observation period which followed the cessation of biting. This may be an example of proguanil failing as a causal prophylactic at this dose (300 mgm. once weekly) and acting only as a suppressive, but, unfortunately, an unequivocal interpretation cannot be made. It happened that an accident in the laboratory some time before had allowed infected mosquitoes to escape in the infecting room, and he may have been bitten there by one of them after he had taken his last dose of proguanil. He was given treatment with mepacrine (400 mgm. for 2 days) and proguanil (600 mgm. for 8 days) and then, after a month during which parasites were never found, he was subjected to a second experiment. The second experiment lasted 4 weeks, during which 86 infected mosquitoes fed on him. Parasites were not found during this time and during a further 4 weeks observation period. He was then given a susceptibility test and he showed parasites 14 days after being bitten.

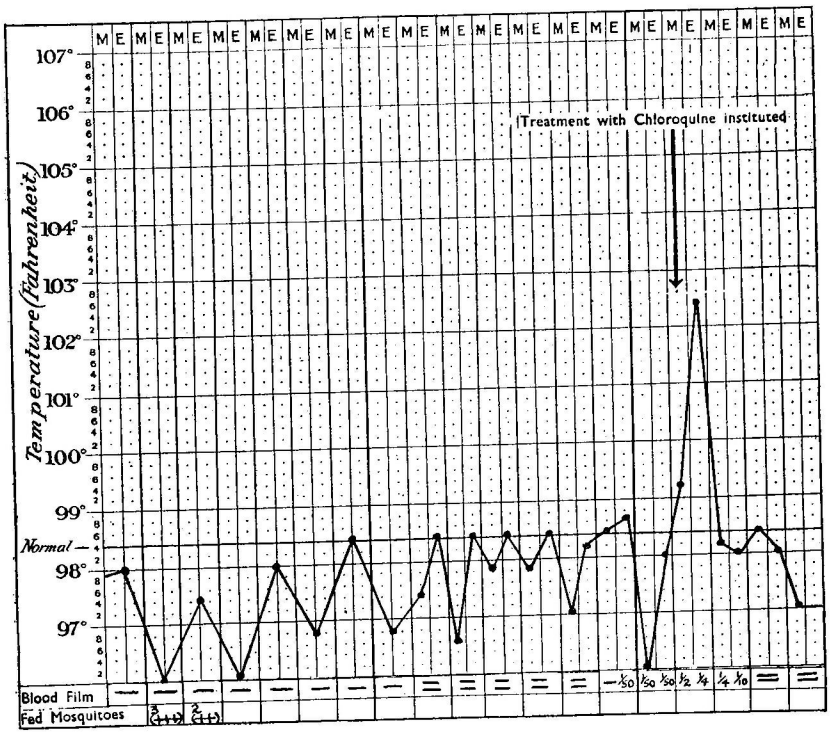
Of the remaining nine volunteers in this group, two, through circumstances beyond our control, could not be given a susceptibility test. The seven who were tested responded satisfactorily.

Discussion :

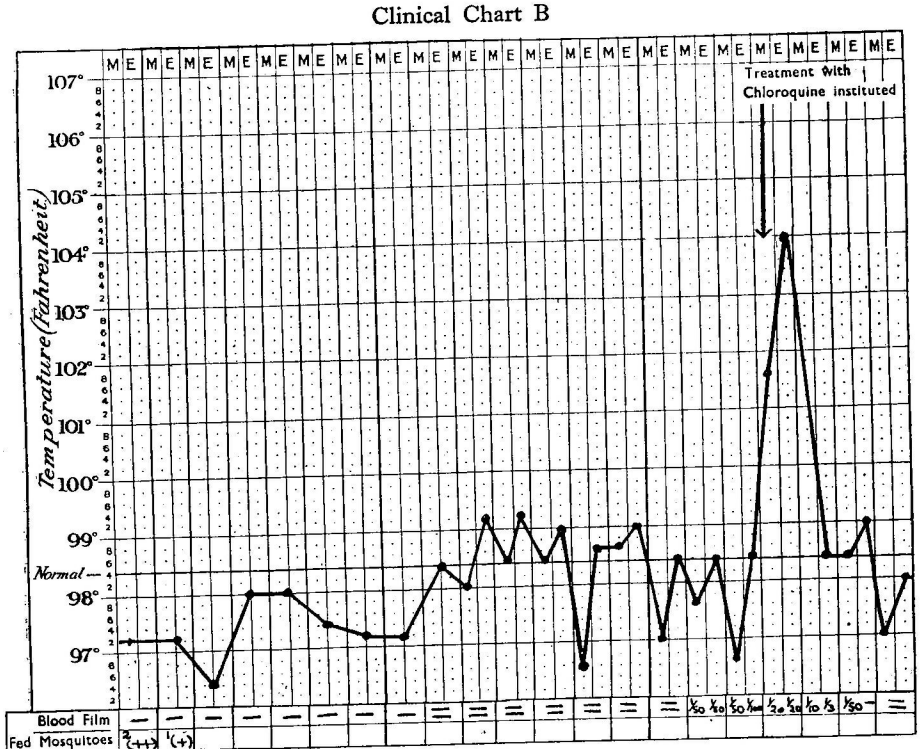
Of the 35 volunteers used in these experiments to test the efficacy of various protective treatments with proguanil, two volunteers were possibly imperfectly protected. In the case of the first, a volunteer receiving 0.1 gramme twice weekly, the drug may have failed both as a causal prophylactic and a suppressive, but for reasons

/already given ...

Clinical Chart A

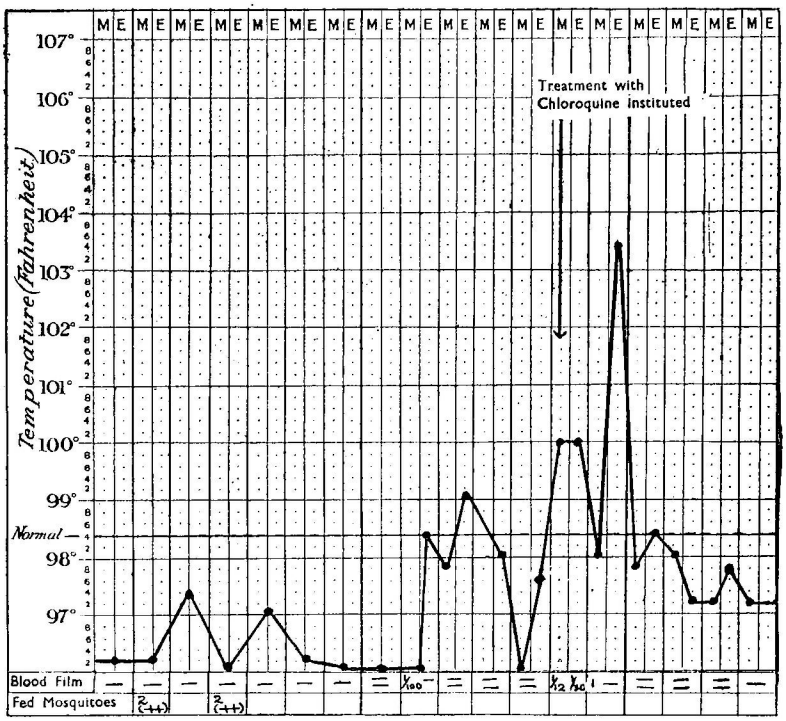


Clinical Chart B

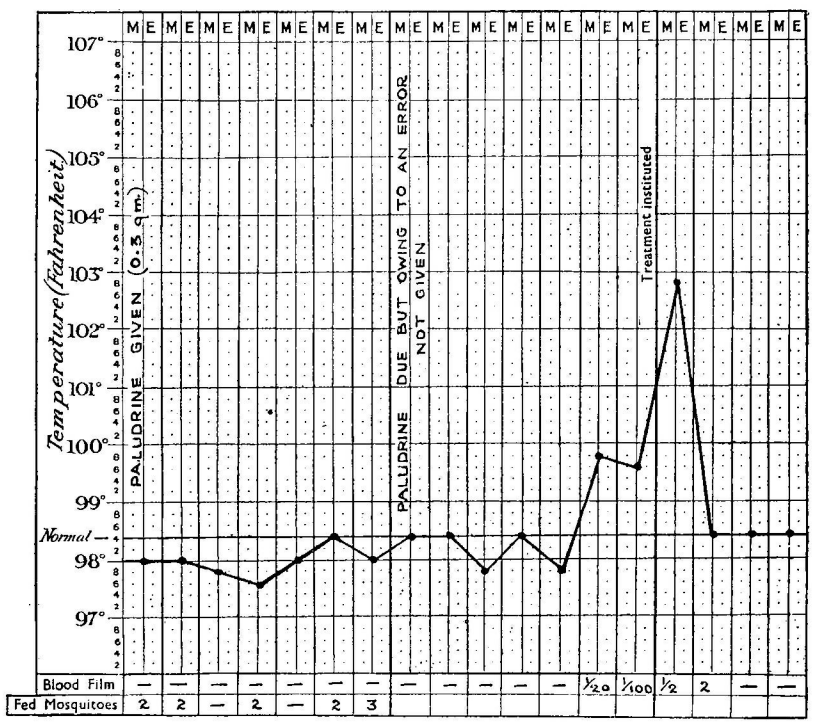


Note :—In all the charts the density of the parasites in the blood films is quoted as parasites per oil immersion field (1/12″ objective, x 6 oculars) in a thick blood film. Mosquitoes are described as positive (+ +) when dissection showed a large number of sporozoites.

Clinical Chart C



Clinical Chart D



Note :—In all the charts the density of the parasites in the blood films is quoted as parasites per oil immersion field (1/12″ objective, x 6 oculars) in a thick blood film. Mosquitoes are described as positive (+ +) when dissection showed a large number of sporozoites.

already given we think that failure of the drug is not the explanation. Instead, in some way or another, medication was avoided. The second possible failure, in a volunteer receiving 0.3 gramme once weekly, may be an example of proguanil acting only as a suppressive at this dose, but again there were circumstances which preclude an unequivocal interpretation being given. The element of doubt regarding the true explanation of each case is to be regretted, but anyone who has conducted extensive tests with human volunteers will know how extremely difficult it is to achieve complete and absolute control of them at every stage for a long period.

In the remaining 33 volunteers proguanil gave apparently perfect protection against falciparum malaria. Susceptibility tests, however, showed that one of the volunteers was apparently completely immune, and that three had a considerable degree of immunity. The tests could not be done on two others. These various facts are summarized in Table II.

There were, then, 27 volunteers in whom the drug apparently received a real test. How rigorous it was depends, of course, on the measure of immunity possessed even by these volunteers whom we proved susceptible to the parasite, and, unfortunately, there is no absolute measure of immunity. We ourselves, however, have been surprised by the severity of the malaria induced in many of the subjects selected for our work, and we think that the test we have offered to proguanil has been generally a rigorous one. We have, whenever possible, avoided making our volunteers experience unnecessary discomfort, and it was our purpose when performing the susceptibility tests to institute treatment as soon as the presence of parasites was definitely confirmed, which meant finding undoubted parasites in fair numbers on two successive occasions. Sometimes, however, quite severe symptoms developed before we had satisfied our requirements concerning parasites. Three of the clinical charts (A, B, C) reproduced illustrate this. Another example of the "reality" of our test

/was proved ...

TABLE III

Prepatent periods obtained in susceptibility tests.

Prepatent periods : in days	9	10	11	12	13	14	15	16	17	18	19	20
Number of volunteers with the indicated prepatent period	1	2	6	2	9	2	2	2				1

was proved by an accident. A volunteer in Group 3 (he does not appear in the experiment proper described above because he was replaced) had been subjected to 6 weeks' biting during which he had received six weekly doses of proguanil 0.3 gramme. Nothing of note occurred during this period. Then, owing to an error, his seventh dose was not given. He was bitten on four occasions after his last dose of drug, and he developed malaria 13 days after the first of these occasions. His clinical chart is shown as case D.

Further evidence that our test has been a sound one is presented in Table III. This table summarizes the prepatent periods (time from the first biting to the appearance of parasites) encountered in our susceptibility tests. A few of them are prolonged, but the majority fall within what are usually regarded as normal limits.

SUMMARY

African volunteers to whom proguanil was being administered in various dosage regimes were exposed almost daily for 2 months to the bite of Anopheles gambiae infected with Plasmodium falciparum. The dosages used were 0.1 gramme daily, 0.1 gramme twice weekly, and 0.3 gramme once weekly. With two doubtful exceptions complete causal prophylaxis was obtained.

SECTION THREE

AN EXPERIMENT TO INVESTIGATE THE PROPHYLACTIC VALUE OF PROGUANIL AGAINST A STRAIN OF Plasmodium falciparum KNOWN TO BE RESISTANT TO THERAPEUTIC TREATMENT

The ability of malarial parasites to develop resistance to proguanil was first demonstrated with Plasmodium gallinaceum in chicks (Bishop & Birkett 1947; Williamson, Bertram & Lourie 1947). Further demonstrations followed with P. cynomolgi in monkeys (Hawking & Perry 1948; Schmidt et al. 1949), and finally it was shown by Seaton and Adams (1949) that P. falciparum and by Seaton and Lourie (1949) that P. vivax could be made resistant in man. The early literature has been reviewed by Bishop (1951) and subsequent papers by Goodwin and Rollo (1955) and Covell et al. (1955).

The early demonstrations were all made under extremely artificial conditions. Infections in which parasites were numerous and multiplying freely were first treated with amounts of proguanil that barely impeded their progress in any way, and then, during successive passages of the infection, the amount of drug used in the treatment was progressively increased. Under such conditions, resistance usually appears in about three months. It was also shown that the drug-resistance survived the sexual cycle in the mosquito. Resistance was measured in these experiments by the response of the blood parasites to treatment, but it can be demonstrated, at least in the case of P. gallinaceum, that a resistance developed by the blood parasites is transferred to all stages in the life history. Thus, proguanil is without causal prophylactic action against a strain to which the blood parasites have been made very resistant (personal observations), and such

/a strain ...

TABLE IV

Results from the Tampin Clinic, Malaya, on the Treatment of Malaria with proguanil.

P.falciparum.

Year	Dosage	No.cases treated	No.failures
1947-8	100 mg. x 1	26	0
1947	300 mg. x 1	34	0
1947	300 mg. x 5-7	32	0
1948	300 mg. x 1	26	0
1948	250 mg. x 1	15	4
1948	300 mg. x 5-7	2	0
1949	250 mg. x 1	43	10
1949	300 mg. x 1	31	7
1949	500 mg. x 1 250 mg. x 4	5	1
1949	300 mg. x 5-7	24	11
1950	300 mg. x 5-7	69	34
1951	300 mg. x 5-7	32	20
1952	300 mg. x 5-7	23	11
1952	400 mg. x 5-7	20	12
1952	600 mg. x 5-7	28	12

P.vivax.

1947-49	300 mg. x 1	31	0
1947-49	300 mg. x 5	9	0
1948-49	100 mg. x 1	17	0
1950	300 mg. x 5	4	0
1951	300 mg. x 5-7	36	2
1952	300 mg. x 5-7	11	1

a strain can also be transferred to the mosquito from chicks still receiving drug and normal development of sporozoites will occur. (Bishop and Birkett 1948).

The apparent ease with which malarial parasites can be made resistant to proguanil under laboratory conditions prompted Field and his colleagues in Malaya to look for resistance amongst a mixed population of Malayans and Chinese who had been given proguanil, probably in irregular fashion, for some years. This they did at their experimental malaria clinic in Tampin. When proguanil was first introduced into Malaya (late 1946) overt attacks of malaria in the natives were successfully controlled at this clinic with single doses of 100 mgm. Success with this treatment continued to be achieved for more than a year, but then, in October 1948, occasional patients were encountered who failed to respond to the single dose treatment, although a more extended treatment was effective. Still later (early 1949), cases were obtained which failed to respond to full therapeutic treatment (300 mgm. to 600 mgm. a day for 7 days). Treatment was described as failing if parasites were still present on the seventh day or if earlier interference with another drug was necessary. Resistance was first found with falciparum malaria and later with vivax malaria. A summary of all the results obtained in the Tampin clinic up to November, 1952, which were received through the courtesy of Dr. J. Field, Dr. T. Wilson and Dr. J. F. Edeson is given in Table IV. (See also Edeson & Field 1950; Wilson, Munro & Richard 1952; and the annual reports of the Institute for Medical Research, Malaya, for 1951-1954).

It is an interesting fact that throughout the years when resistance to proguanil was clearly developing there was no indication that the drug was failing appreciably as a prophylactic. In other words, the incidence of malaria on the plantations where it was being used continued to remain low and, consequently, one is tempted to think that the resistant strains discerned in

/individuals ...

individuals were strains which developed in them themselves, and were not necessarily transmitted to them.

The results of Field and his colleagues, however, caused some disquiet among the Army authorities because of the number of troops then operating in malarious areas, and it was, therefore, decided that experiments should be done to determine the hazards involved for personnel taking proguanil for prophylaxis in areas where strains exhibiting resistance to treatment with this drug might be carried by mosquitoes.

Experimental :

The work was made possible through the co-operation of the Medical Research Institute in Malaya who sent a proguanil-resistant strain of P. falciparum to Nairobi, and of the War Office who allowed British volunteers of the East African Command to be used.

The strain sent from Malaya had resisted a full therapeutic treatment with proguanil (400 mgm. daily for more than six days) in the Chinese patient from whom it was taken. It was dispatched as parasited blood which was kept cold with ice in a thermos flask, and in Nairobi the blood was injected into patients for whom fever therapy had been prescribed. One of them proved a suitable gametocyte carrier and a number of Anopheles gambiae was fed on him. When the mosquitoes became infected they were used for the attempted infection of ten British Army volunteers.

The majority of the volunteers had only recently arrived in East Africa and none had any history suggesting he ever had malaria. It was planned to expose them to infected mosquitoes daily for 28 days. The prophylactic treatment given was 100 mgm. of proguanil daily, starting on the first day of exposure to infection and continuing for 14 days after the last exposure. A total of 42 doses was therefore administered to each volunteer who completed the course. The volunteers were then observed for a further six weeks.

A total of approximately 50 mosquitoes fed on each man. They were seen to probe on several occasions before engorging; often, nine or ten wheals would be seen after the feed although only two mosquitoes out of a pot of four may have engorged. As sporozoites are contained in the saliva, and some saliva is probably injected at each time of probing, the infection to which these volunteers were exposed was very heavy. Dissections were made of 213 mosquitoes from the batches used, and 91 per cent were found to be infected. Five controls were used at different times in the experiment, each being bitten by two mosquitoes, and all developed overt malaria.

Results :

Six of the ten men were protected and completely fit throughout the twelve week period of experiment and observation. One man showed parasites on the 15th day and developed overt malaria on the 17th day. Two men showed parasites on the 27th day and developed overt malaria on the 28th and 30th days respectively.
A fourth man showed parasitaemia on the 45th day, three days after proguanil administration had ceased, and was treated with chloroquin at once.

Discussion :

The experiments described above were planned in 1951 because of the anxiety amongst those responsible for the health of the troops and of the workers on the estates in Malaya which was being aroused by talk about proguanil resistance. They were completed in 1952 but, for various reasons, the results could not be published immediately. They formed part of a larger programme of work, part of which will shortly be described in a series of papers, and incidental to them it was shown that one strain, at least, of P. falciparum resistant to proguanil - the strain used in these experiments - was also resistant to pyrimethamine (Robertson, Davey & Fairley 1952).

Anxiety concerning resistance to proguanil is now being voiced again. Laing (1956) has been able (as we were) to infect mosquitoes by feeding them on patients carrying a strain of P. falciparum resistant to proguanil while the drug was still being administered and he concludes : "This extension of proguanil resistance to gametocytes of P. falciparum may seriously detract from the usefulness of the drug, as proguanil-resistant strains can thereby be directly transmitted by the mosquito. In Malaya, proguanil is still extensively used as a suppressive drug, particularly by Military and Police forces; its use in the future in preference to other antimalarial drugs would appear to have an element of uncertainty."

It seems to us that the implications in this statement err on the side of pessimism. Proguanil was introduced into Malaya in 1946; resistance could be demonstrated to exist in some people in 1948 (see Table IV) and perhaps in a somewhat higher proportion subsequently. But even now, almost ten years later, there is no evidence that proguanil fails appreciably as a prophylactic drug in the territory, and it continues to be used by large numbers.

Why resistance has not caused proguanil to fail more is a matter for surmise with our present knowledge. It appears probable that resistant strains arise in individuals who practise very irregular prophylaxis or who treat themselves inadequately when they feel feverish. Thereafter, the transmission of the strains may proceed only slowly because adults are often poor "carriers". Finally, as we have shown above, the exo-erythrocytic forms are more susceptible than the blood forms, and a strain exhibiting marked resistance therapeutically may be controlled in great measure prophylactically. It must also be of importance that where proguanil is used prophylactically, the use of other drugs, such as chloroquin, is advised for treatment, and resistant strains or incipient resistant strains may thereby be eliminated.

CONCLUSIONS AND SUMMARY

1. A strain of *Plasmodium falciparum* which, for all practical purposes, was completely resistant to the therapeutic action of proguanil was controlled in large measure prophylactically through the action of the drug on exo-erythrocytic forms.

2. The test to which proguanil was subjected was a most stringent one - there can be few malarious areas where so many *infective* bites would be given daily - and it is clear, therefore, that before there can be a wholesale breakdown of the protection given by the prophylactic use of proguanil, the strain must be very resistant and the transmission rate very high.

SECTION FOUR

THE PROPHYLACTIC EFFECT OF 50 mgm. OF CHLOROQUINE BASE DAILY IN BRITISH TROOPS EXPOSED TO INFECTION WITH *Plasmodium falciparum*

Commencing in 1948, evidence began to accumulate that in the Tampin area of Malaya larger doses of proguanil were required in the treatment of infections with *P. falciparum* and *P. vivax* in the indigenous population than had been required when the drug was first introduced, and it was concluded that strains resistant to it were emerging. (See the reports of the Institute for Medical Research, Malaya, for 1951 et sec.) When these results became known, people expressed some anxiety that, if the resistant strains spread, proguanil might become less effective as an antimalarial prophylactic not only on the rubber estates in Malaya but also for the armed forces then operating extensively in the territory. Subsequent history in Malaya gave no indication that resistance to proguanil was in fact spreading, or at least there was no indication that proguanil was failing to give very effective protection against malaria to workers on the estates and to the troops of the Imperial Army. Actually, the incidence of malaria amongst service personnel in Malaya during the recent emergency is probably the lowest ever recorded for any force operating in a malarious area.

However, when the outcome in Malaya regarding the efficacy of proguanil was still uncertain, it was considered wise to make preparations for an alternative drug to be used in the armed forces, should the need ever arise. The obvious alternative was chloroquine but, apart from the obversations of Fairley (see below), no

/information ...

information was available regarding the use of chloroquine in daily doses as a suppressive, and it is a daily dose which the Imperial Army will wish to use.

Experimental :

Fairley (1946) reported that in volunteers heavily infected with P. falciparum (they were each given 17-18 infective bites) symptoms of malaria failed to develop and parasites were never found in thick blood smears whilst they were taking chloroquine in doses of 0.1 gramme daily or subsequently. Similar results were also obtained in a few volunteers with dosages of 50 mgm. of chloroquine daily.

Further tests of the lower dose were desired, and arrangements were therefore made to expose non-immune British Army volunteers to the bites of mosquitoes (Anopheles gambiae) infected with P. falciparum. Twenty volunteers were used. The dose investigated was 50 mgm. given daily from the first day of exposure until 14 days after the last day of exposure. No loading dose was included because we thought it important to determine if it could be avoided. In these days of rapid air travel and sudden preparations, it would be of great administrative convenience if antimalarial prophylaxis with chloroquine, as with proguanil, need not be started till the day of arrival in a malarious area. There is, possibly, one slight disadvantage in starting treatment on the first day of arrival in a malarious area. Any symptoms of fatigue, headache or alimentary upset resulting from the journey may be wrongly attributed to the antimalarial drug which has been taken for the first time on that day. This association may subsequently lead to some reluctance to take the drug regularly.

Sufficient mosquitoes were not available to give all the volunteers an intensive infection simultaneously. Three groups were therefore formed and the tests carried out at different times. During the first week,

approximately 60 mosquitoes bit each volunteer; in subsequent weeks, about 30 were used on each man. The infection rate of the mosquitoes was between 33-40 per cent. The mosquitoes were infected with a specially selected Malayan strain of P. falciparum known to be resistant to proguanil, or one of the two East African strains that we call the Mombasa strain and the Lake Victoria strain.

The volunteers were bitten intensively on the first day of infection, so that the challenge would be particularly severe at the beginning of the test when there had been little time to build up a high concentration of chloroquine within the body. If the drug could resist the challenge at this early period, then, clearly, it could be expected to be successful throughout any subsequent period.

The groups were arranged as follows :-

Group 1. Eight volunteers were infected for six weeks with both the Malayan No. 2 strain and the Mombasa strain. Daily treatment was given for eight weeks.

Group 2. Eight volunteers were infected for four weeks with the Lake Victoria strain. Daily treatment was given for six weeks.

Group 3. Four volunteers, although infected for only one week, were all given an intensive infection during that week with the Mombasa strain. Daily treatment was given for three weeks.

For interest, two other volunteers were exposed to infection with the Lake Victoria strain for three weeks, but given proguanil. They were each bitten by a total of approximately 65 mosquitoes of which at least 33 per cent were infected. 100 mgm. of proguanil was given daily, starting on the first day of exposure and continuing for seven days after the last day of infection. An observation period of six weeks was then made.

Results :

All the volunteers were protected throughout the experimental period from major malarial symptoms. A few men taking chloroquine had minor symptoms ,

(temperatures up to 100-101°F. in two cases, shivering, joint pains, tender liver and slight headaches) some 8-14 days after infection commenced, but no malarial parasites could be found in spite of persistent searching of thick smears. Apart from the routine daily smears, several additional smears were taken throughout the day from any volunteers about whom there was any uncertainty. Neither of the two volunteers taking proguanil exhibited even minor symptoms.

The symptoms amongst the men taking chloroquine were possibly due to the abnormally intense infection to which the volunteers were subjected. Because chloroquine acts only as a schizonticide, the parasites pass unhindered through the early exo-erythrocytic stage in the liver, occurring during the first week, and are killed by the drug when they invade the blood stream during the second week. The intense infection used must have resulted in very large numbers of parasites being killed in the blood stream, and it is thought likely that the disintegration of such large numbers of parasites was the cause of the transient symptoms which disappeared without any increase of dose. It is also possible that the exo-erythrocytic forms in the liver may produce some minor symptoms.

CONCLUSIONS AND SUMMARY

A 50 mgm. tablet of chloroquine base taken daily acted as a satisfactory suppressive for 20 non-immune volunteers infected with one or more of three strains of P. falciparum. They were protected from all major malarial symptoms and no parasites were ever found. The volunteers were exposed to a very intensive initial infection, followed by a lighter infection for one to six weeks. The large number of parasites liberated in the blood at the end of the first week and their subsequent destruction by the drug, or the exo-erythrocytic forms of the parasite in the liver, were considered responsible for minor malarial symptoms occurring in several of the volunteers 8-14 days after the start of the experiment. Two men who received 100 mgm. of proguanil daily were fully protected, and did not show even minor symptoms.

SECTION FIVE

EXPERIMENTS WITH CHLOROQUINE AND AMODIAQUINE IN PROPHYLAXIS

It is well established that 300 mgm. of chloroquine base once weekly is a reliable suppressive treatment for all types of malaria. Doses of amodiaquine, such as 400 or 600 mgm., have been recommended for use at fortnightly intervals on the results of field trials (Singh et al. 1953; Edeson et al. 1955), but there is no evidence to suggest that chloroquine in fortnightly doses would be in any way inferior. We considered it important to obtain a comparison of the activity of the two drugs under identical and controlled conditions, and also to establish how low a dose of either might be used to give a considerable degree of protection to people likely to have some degree of immunity, such as plantation workers. Immunity to malaria in man seems to be directed mostly, if not quite entirely, against the blood forms, and therefore this immunity will assist the suppressive action exerted by schizonticides such as chloroquine or amodiaquine, and so a lower dose of drug can be effective as a suppressive in partially immune subjects than is required for non-immune subjects.

Experiment I :

Anopheles gambiae infected with P. falciparum were allowed to feed on a group of ten Africans and were infected in the same way as we have described previously. The volunteers were exposed to infection almost daily for 35 days. A total of approximately 80 mosquitoes fed on each volunteer, and of these 88 per cent were calculated to be infective (557 mosquitoes were dissected to obtain

/this estimate).

this estimate). The strain used was one quite foreign to the volunteers as it was the Malayan Strain No. 2 previously described and selected as being a proguanil-resistant strain of P. falciparum. The dose used was 150 mgm. of chloroquine base once weekly, which is half the usually recommended dose. To allow the drug concentrations to reach a 'steady state' before the challenge began three doses at weekly intervals were given prior to the first bite. Three doses were also given after the last bite to attempt eradication of the infection. Altogether, then, a total of eleven doses were given at weekly intervals. An observation period of at least 63 days after the last dose was made before tests of the susceptibility of the volunteers to malaria were carried out.

Results : All the volunteers were free from overt malaria and fit for work throughout the experimental period. One man showed very scanty parasites in a thick film on one occasion. There was no evidence that this volunteer was unwell and he did not exhibit a morning or evening pyrexia. Susceptibility tests were carried out on seven of the volunteers who were still available, and all were shown to be capable of being infected. Their incubation periods were,respectively, 11, 12, 12, 12, 13, 13, and 17 days.

Experiment II :

Ten volunteers were exposed for 35 days to the Malayan No. 2 strain in exactly the same way as described in Experiment I, but now the dose given was 300 mgm. of chloroquine base once fortnightly. Two doses at fort-nightly intervals were given prior to infection and two subsequent to infection, making a total of six doses in all.

Results : All the volunteers were free from overt malaria throughout the experimental period. One man was unwell for one day but, almost certainly, all his symptoms were due to a very mild respiratory infection.

TABLE V

Chloroquine as a suppressive in doses of 150 mgm. weekly.

No.of volunteers	Exposure period days	No.of doses prior to exposure	No.of doses subsequent to exposure	Total No.of Strain doses	Approx No.of mosquitoes biting each vol.	No.dissected & % + of batches used	Result		Susceptibility test. Incubation period in days.
							protected	not protected	
10	35	3	3	11 Malayan No.2	80	557 88%	10 but 1 man showed scanty parasites on one occasion but no symptoms	0	11 12 12 12 13 13 17

Chloroquine as a suppressive in doses of 300 mgm. fortnightly.

10	35	2	2	6 Malayan No.2.	80	557 88%	10 though 1 man was not well for one day, probably cough	0	11 12 17 15 19

Several additional thick blood films were taken from him on that day, and on the next, but no parasites were seen.

Susceptibility tests were carried out later, and all the volunteers tested were shown to be susceptible to the strain. The incubation periods of those available to be tested were 11, 12, 15, 17, and 19 days respectively.

The results of Experiments I and II are summarised in Table V.

Comment : These tests do not reveal any significant difference in activity between the two suppressive regimes. A dose of 150 mgm. once weekly, or 300 mgm. fortnightly, administered regularly, gave a very useful degree of protection to subjects likely to have some slight degree of immunity, even though the intensity of the infective challenge was high.

It might have been thought that five weeks' exposure to intense infection and the destruction of numerous schizonts by schizonticide drugs would have stimulated the immunity mechanism of these volunteers. They might be expected to have acquired a considerably greater degree of immunity than those exposed to a similar challenge but protected by a causal prophylactic drug. Causal prophylactic drugs eradicate the early exo-erythrocytic forms before they can enter the blood stream and stimulate the immunity mechanism. I have studied the prepatent and incubation periods of these men who had been protected by a suppressive drug and the incubation and prepatent periods of those groups we have tested for susceptibility who were protected by causal prophylactic drugs such as proguanil and 5943. (Thomas, Robertson & Davey 1953; Robertson 1957). I can find no significant difference between the two groups. There is no evidence to suggest that in a short period, such as the period of this test, a more significant degree of immunity is built up by the group using a schizonticide as a suppressive drug, than by the group using causal prophylactic drugs which prevented the parasites from ever reaching the erythrocytic phase.

Experiment III :

In this third experiment, a lower dose of chloroquine was used and its efficiency compared with that of the same dose of amodiaquine. This time, the East African Mombasa strain of P. falciparum was used. The challenge to the volunteers was considerably less severe than in previous tests because of a shortage of infected mosquitoes at one period. Some 85 mosquitoes fed on each man. Of the batches used, a sample showed sporozoites in 38 per cent (184), and even these were not so heavily infected as had been found in other experiments.

Two groups, each of four African volunteers, were bitten over a period of 56 days. One group was given 100 mgm. of chloroquine base weekly, and the other 100 mgm. of amodiaquine base weekly. To allow the blood concentrations of the drugs to reach a 'steady state' before the attempted infection began, the volunteers were given three doses of 100 mgm. at weekly intervals prior to the exposure period. Treatment was continued for two weeks after the last time of biting, making a total of 13 doses.

Results : In the chloroquine group three men were protected and one man showed parasitaemia on Day 33. In the amodiaquine group two men were protected and the other two had mild malaria symptoms, one of them showing occasional parasitaemia.

Susceptibility tests were made later, and the incubation period in days of the volunteers available for testing were 7, 11, 21 for the chloroquine group, and 11, 12, 15 for the amodiaquine group.
(Due to a mistake, the man with a 7-day incubation period was bitten by some 30-40 infected mosquitoes instead of the 2-4 usually employed for a susceptibility test.)

TABLE VI

Chloroquine - 100 mgm. weekly.

No.of Vols.	Exposure period days	No.of doses prior to exposure	No.of doses subsequent to exposure	Total No.of doses	Strain	Approx No. of mosquitoes biting each vol.	No.dissected & % + of batches used	Result: protected	Result: Not protected	Result: Day on which parasitaemia developed	Susceptibility test. Incubation period in days
4	56	3	2	13	Mombasa	85	184 38%	3	1	33	7 11 21

Amodiaquine - 100 mgm. weekly.

4	56	3	2	13	Mombasa	85	184 38%	2	1 mild symptoms; 1 definite symptoms on 2 occasions and occasional parasites seen.	-	11 12 15

Chloroquine (base) - 250 mgm.fortnightly.

4	49	2	2	8	Mombasa	60	184 38%	3	1 symptoms & spleenic enlargement but no definite parasitaemia	-	11 13 15

Amodiaquine - 250 mgm. fortnightly.

4	49	2	2	8	Mombasa	60	184 38%	4	0	-	17

Experiment IV :

This experiment was carried out at the same time and in exactly the same way as Experiment III above, but the two groups were given chloroquine and amodiaquine at fortnightly intervals. Doses of 250 mgm. (base) of the drugs were given and the exposure period was 49 days. Two doses were given prior to infection and two doses subsequent to infection, a total of eight doses in all being given.

Results : Three of the men on the chloroquine regime were free of parasites and symptoms. The fourth had some malarial symptoms and developed a slightly enlarged spleen, but definite malaria parasites could never be detected. The four men on the amodiaquine regime were free of parasites and symptoms.

Summary of results of Experiments Nos. III and IV are in Table VI.

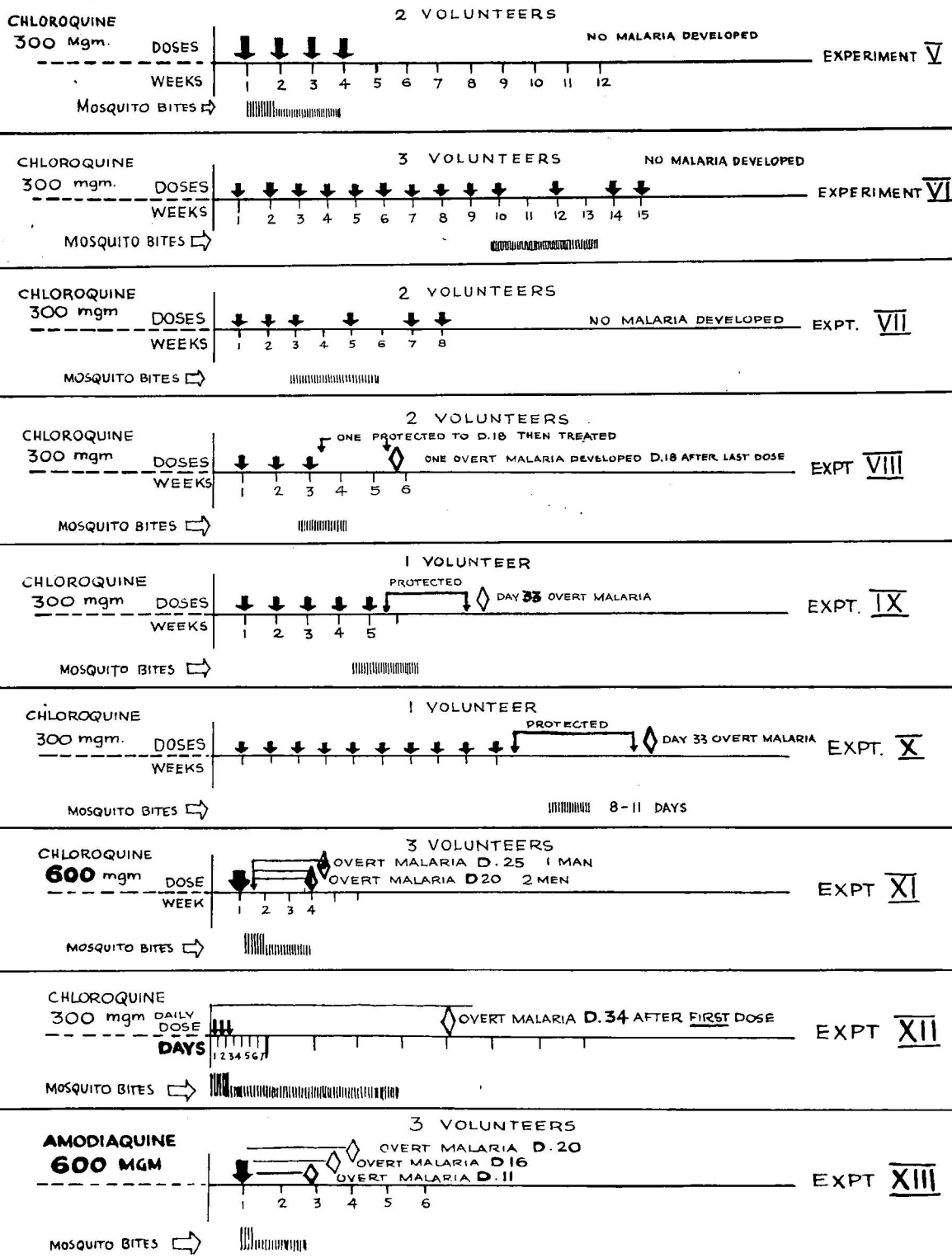
Experiments Demonstrating the Effective Suppressive Blood Levels and Persistence of Chloroquine and Amodiaquine in Non-immune Volunteers :

300 mgm. of chloroquine base has in many areas been considered a suitable weekly prophylactic regime for non-immune subjects. We wished to find out if there was much margin with this dose, so, in addition to the tests described above, we carried out some tests using three strains of P. falciparum which were available to us - our Mombasa and Lake Victoria strains, collected at random, and Malayan No. 2, specially selected as showing resistance to proguanil. There is little published evidence to suggest that strains exist which are poorly susceptible to chloroquine, other than one note by Messent (1951) from West Africa, and it has not been found possible, even in the laboratory, to induce chloroquine resistant Plasmodia, so the chance of

/encountering strains ...

TABLE VII

Summary of results obtained in Experiments V – XIII



encountering strains truly resistant to chloroquine under natural conditions appears remote. It was only after some ten years' use of mepacrine that a strain of reduced sensitivity to mepacrine was clearly shown to exist, the Aitaipe-Wewak strain. (Fairley 1946). Experience with other antimalarial drugs suggests that casual reports of such strains being found should be treated with great suspicion until the possibility of a technical error has been very carefully excluded.

Experience with chloroquine suggests that a very adequate margin of effectiveness is present in the dosage regimes usually recommended and used at present, both for suppression and treatment, e.g. the three and four day treatment courses of 1.2 and 1.5 gramme. We think that in underdeveloped countries where chemotherapy may be severely limited because of expense, rather lower doses of chloroquine could frequently be used with little additional risk, particularly in the many cases where the diagnosis of malaria may be unlikely but, nevertheless, antimalarial treatment has to be given as a safeguard. This is clearly shown by Chaudhuri et al. 1950; Singh et al. 1953; Wilson & Edeson 1954; Hockenga 1954; and Jeffrey et al.1956.

The tests described below, and shown on Table VII, were carried out from time to time when non-immune European volunteers and infected mosquitoes were available. These tests were planned to establish, after a certain dose, the period of persistence of a blood level of chloroquine sufficient to suppress parasitaemia and prevent the parasites multiplying to produce overt malaria. This was done either by giving a loading dose or giving a number of weekly doses so that what might be considered the chloroquine 'steady state' was reached. The volunteers were exposed frequently to the bites of mosquitoes infected with P. falciparum so that, some six days later, erythrocytic forms were being discharged constantly into the blood and being killed or suppressed by the chloroquine present in

/the blood ...

the blood. Doses were then omitted but the biting continued. As the drug level fell away, a point was reached where the chloroquine could no longer suppress the erythrocytic forms but they survived and multiplied so that overt malaria resulted. Provided the intensity of infection did not vary greatly, this period between the last dose of drug and the onset of overt malaria can be used to compare the excretion rate and the effectiveness of different drugs, and also any variation of strains to drugs of the purely schizonticidal type.

We were not always able to ensure a steady and prolonged exposure period to infected mosquitoes, but, when there was a shortage of mosquitoes, we timed the biting period to ensure that erythrocytic forms were being discharged into the blood well before and after what experience led us to consider were the critical periods.

Once again, also, we attached rather more importance to a positive result with the development of overt malaria than to a negative result where malaria was prevented. The number of volunteers on each of these experiments was small and the exposure period was not always prolonged; nevertheless, the results obtained by this method give information which could usefully be amplified by others working on the chemotherapy of malaria with non-immune volunteers.

We show below that two non-immune volunteers put on to 300 mgm. of chloroquine base weekly on the day exposure to infection started were protected from malaria, in spite of receiving no loading dose or build up. (Experiment No. V). Again, in three volunteers, after a build up of ten weekly doses of 300 mgm. of chloroquine base, the doses on two alternate weeks were missed without malaria developing, (Experiment No. VI), and this was also found to occur after only three weekly doses had been given to two volunteers and the doses for the fourth and sixth weeks were omitted. (Experiment No. VII).

Additional tests were made with non-immune volunteers to demonstrate how long an effective suppressive blood level of chloroquine would persist. It was found that when exposure was commenced after three weekly doses of 300 mgm. of chloroquine, overt malaria developed in one man eighteen days after his last dose of drug. (Experiment No. VIII). Normally, since one would have expected overt malaria to develop 8-12 days after the first exposure to infection, we conclude that sufficient concentration of drug persisted in the blood to kill or prevent multiplication of parasites until just before that eighteenth day. Another volunteer, after five weekly doses of 300 mgm. of chloroquine, was then bitten daily for a week, and parasitaemia developed 33 days after his last dose of chloroquine. (Experiment No. IX). Similarly, with another volunteer, after ten weekly doses of 300 mgm. of chloroquine base, 33 days elapsed after his last dose before parasitaemia became evident. His period of exposure to infection was limited to the eighth to the eleventh day after his last dose of drug because of shortage of mosquitoes. (Experiment No. X).

As I have mentioned above, experience suggested that there was little point in exposing any earlier as parasites appearing in the blood earlier would have been suppressed by the level of chloroquine present then. In this experiment, when exposure was on the eighth to eleventh days after the last dose of drug, one would have expected overt malaria to have developed some 8-12 days after the exposure, if an inactive or non-persistent drug had been given. In fact, overt malaria did not develop until 33 days after the last dose of drug had been given. We consider this delay was due to the suppressive action of the chloroquine, and only as the blood level of chloroquine slowly diminished were the parasites entering the blood able to survive and eventually multiply to cause overt malaria.

In Experiment No. XII it was found that after a single dose of 600 mgm. of chloroquine and subsequent almost daily exposure to infection, overt malaria developed in two of the volunteers on the twentieth day after dosing, and on the twenty-fifth day in the third volunteer.

Experiment V :

Two non-immune volunteers were given 300 mgm. of chloroquine base weekly without any loading dose, and biting was started on the day of the first dose and continued for three weeks. The biting was heavy, six infected mosquitoes on the first day, but became lighter (one or two infected mosquitoes almost daily) during the next eighteen days. The Mombasa strain was used. A final dose of chloroquine was given four days after the last bite, making a total of four doses.

Result : Both volunteers were protected and, in spite of a follow-up of ten weeks, no malaria developed during that time.

Experiment VI :

Three men were dosed at weekly intervals for ten weeks with 300 mgm. of chloroquine base. Between the ninth and tenth dose (the sixtieth day) biting was started with an intensive bite on that day from, approximately, ten infected mosquitoes, and continued with bites every second or third day from two or three infected mosquitoes until the eighty-eighth day. The Lake Victoria strain was used. The doses due for the eleventh and thirteenth weeks were omitted. The normal dose of 300 mgm. was resumed for the fourteenth and fifteenth weeks, after which the experiment was terminated.

Result : All three volunteers were protected throughout the experiment.

Experiment VII :

Two volunteers were given three weekly doses of 300 mgm. of chloroquine base. Two days before the third dose was due they were each exposed to infection with, approximately, five infected mosquitoes and biting with one or two infected mosquitoes continued almost daily for three weeks. The doses for the fourth and sixth weeks were omitted. The normal doses for the fifth, seventh and eighth weeks were given, after which the experiment was terminated.

Result : Both volunteers were protected throughout.

Experiment VIII :

Two volunteers were given three doses of 300 mgm. of chloroquine base at weekly intervals. Two days before they were due to receive their third dose they were bitten by five or six mosquitoes known to be infected with the Lake Victoria strain and continued to be bitten almost daily for ten days by two or three infected mosquitoes. No dose was given for the fourth or fifth week.

Result : Four days after the fifth weekly dose was due (the eighteenth day after the third and last dose) overt malaria developed in one volunteer. It was decided to treat the other volunteer, though still quite fit, on that eighteenth day since his last dose of drug.

Experiment IX :

One volunteer was given four weekly doses of 300 mgm. of chloroquine base. Two days before the fifth dose was due he was exposed to a heavy infection of mosquitoes infected with our Mombasa strain and the biting was continued for nine days. His fifth dose of 300 mgm. was given on the day due, and he was then carefully observed to see when the blood level of chloroquine would diminish to a level that would allow parasites to multiply and overt malaria to occur.

Result : Overt malaria developed on the thirty-third day after his fifth and last weekly dose of chloroquine base.

Experiment X :

A volunteer given ten weekly doses of 300 mgm. chloroquine base was bitten by five infected mosquitoes on the eighth, tenth and eleventh day after his last dose of chloroquine. The strain used was our Mombasa strain.

Result : In this volunteer also overt malaria developed on the thirty-third day after his tenth and last weekly dose of chloroquine.

Experiment XI :

Three other non-immune volunteers were given 600 mgm. of chloroquine base and exposed to a heavy infection (about ten infected mosquitoes) on that day and a light infection (one or two infected mosquitoes) daily for ten days following. The Mombasa and Lake Victoria strains were used.

Result : Two of these volunteers developed overt malaria on the twentieth day, and the third on the twenty-fifth day after the dose.

Experiment XII :

A volunteer was given 300 mgm. of chloroquine base daily for three days, a total of 900 mgm., and he was bitten heavily on the first day of dosing and lightly over the next twenty-eight days with our Mombasa strain.

Result : A suspected parasite was seen on the thirtieth day after the first day of dosing, and overt malaria developed on the thirty-fourth day.

Amodiaquine

Experiment XIII :

Somewhat similar results were found with a 600 mgm. dose of amodiaquine, carried out in the same way and at the same time as Experiment No. XI with chloroquine. One volunteer developed overt malaria on the twentieth day and one on the sixteenth day after exposure to the infection with the Mombasa strain. A third volunteer was infected by the Lake Victoria strain alone and had even developed overt malaria on the eleventh day.

SUMMARY

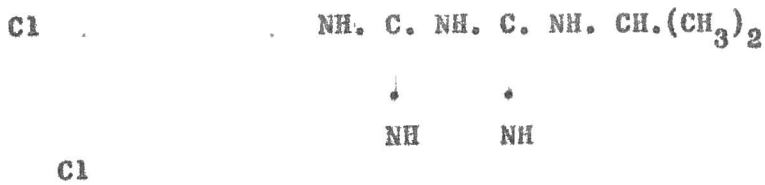
A dose of 150 mgm. of chloroquine base once weekly, or 300 mgm. fortnightly, given regularly, afforded a high degree of protection to 20 African volunteers likely to have a slight degree of immunity who were exposed to a heavy infection with a Malayan strain of P. falciparum. Weekly doses of 100 mgm. of chloroquine and amodiaquine and fortnightly doses of 250 mgm. of chloroquine and amodiaquine were given to four very small groups of African volunteers. These doses all gave a considerable degree of protection to these volunteers in good health and on a good diet. Their infection was rather lighter and was with the Mombasa strain. The results from these few volunteers show that there is no margin, so that considerable symptoms might develop if dosing was at all erratic, intercurrent infection or malabsorption occurred, or if any abnormal strains were encountered.

A biological method of estimating the persistence of an effective blood concentration of schizonticides is described, and a few results in non-immune volunteers are recorded. Several of these tests, involving small numbers of non-immune volunteers, confirm the reliability of 300 mgm. of chloroquine base as a suppressive dose. A few tests with amodiaquine point to it as having no advantages over chloroquine.

SECTION SIX

EXPERIMENTS WITH COMPOUND 5943

Following the discovery of proguanil and during further development work on the biguanides, the compound 5943 (Formula I) was synthesised and found to be more active than proguanil (Curd, Davey, Hendry & Rose 1950). Some clinical work was also done with it (Chaudhuri, Rai Chaudhuri & Dutta 1952; Murgatroyd, F. Personal Communication) which showed that, although effective in smaller doses, its advantages over proguanil were not considerable.



Formula I

At that time, facilities were not available to study its causal prophylactic action, i.e. its action on early exo-erythrocytic forms or its oocyst inhibiting property. It was the main purpose of the present experiments to remedy these omissions.

Action of 5943 on the Early Exo-erythrocytic Forms of Plasmodium falciparum :

Experiment I :

Using our standard methods, a group of six African volunteers were exposed almost daily to

/infection ...

TABLE VIII

COMPOUND 5943 - 20 mg. weekly.

Summary of results of Experiments Nos. 1 & 11.

No.of Vols.	Exposure period days	No.of doses prior to exposure	No.of doses subsequent to exposure	Total No.of doses.	Strain	Approx No.of mosquitoes biting each vol.	No.dissected & % + of batches used.	Result			Susceptibility test. Incubation period in days
								protected	Not protected	Day on which parasitaemia developed	
6	28	0	1	5	Mombasa	70	260 78%	6	0	-	10 12 13 13 14 17

COMPOUND 5943 - 10 mg. weekly.

6	28	0	1	5	Mombasa	70	260 78%	5	1	21	11 11 11 12 13

infection with the Mombasa strain of P. falciparum for a period of 28 days. Each volunteer was bitten by about 70 mosquitoes. Two hundred and sixty mosquitoes from the batches used on this experiment were dissected, and 78 per cent. were found to be infected. The first dose of 20 mgm. of 5943 was given on the first day of exposure to infection (Day 1). This dose was given thereafter at weekly intervals for a total of five doses, the last dose being given on Day 29. An observation period of at least 63 days after the last dose was allowed before susceptibility tests were made.

All six volunteers were completely protected throughout the experiments and no parasites were ever observed in any of them. In subsequent susceptibility tests they were shown not to be immune. The incubation periods for the six men were respectively 10, 12, 13, 13, 14 and 17 days.

Experiment II :

This test was carried out in exactly the same way as Experiment I, except that the weekly dose used was only 10 mgm.

Of the six men exposed to infection, five were completely protected but one developed parasitaemia on Day 21.

Susceptibility tests were carried out in the five in whom no parasites were found and they were shown not to be immune. The incubation period in these volunteers was respectively 11, 11, 11, 12 and 13 days.

The results of Experiments I and II are summarised in Table VIII.

The results described above showed that 5943 had considerable promise as a prophylactic drug, and, therefore, further more detailed studies of it were

/made ...

TABLE IX

SINGLE BITING TESTS - MOMBASA STRAIN.

Time of dosing in relation to biting. Biting time as zero	Dose of 5943	Nos.of volunteers exposed.	Completely protected	Exhibiting overt malaria.
- 168 hours	60 mgm.	3	2	1
	50	6	3	3
	40	1	0	1
	30	1	0	1
	25	1	0	1
- 144 hours	50 mgm.	2	2	0
	25	2	2	0
- 120 hours	50 mgm.	1	1	0
	25	1	1	0
- 96 hours	50 mgm.	2	2	0
	25	4	2	2
- 72 hours	50 mgm.	1	1	0
	25	7	6	1
- 48 hours	50 mgm.	1	1	0
	25	5	5	0
	15	1	1	0
- 24 hours	25 mgm.	2	2	0
	20	2	2	0
	15	1	1	0
0 hours	75 mgm.	1	1	0
	50	5	5	0
	30	1	1	0
	25	4	4	0
	20	6	6	0
	15	10	10*	0
	10	15	14	1
	7.5	5	4	1
	5	3	2	1
+ 48 hours	20 mgm.	2	2	0
	10	2	2	0
	5	1	1	0
	2.5	2	2	0
+ 120 hours	5 mgm.	4	4	0
	1	3	3	0

* Includes 2 non-immunes.

made by carrying out single biting tests as described above using the Mombasa and other strains. In these tests, mosquitoes from infected batches were allowed to bite volunteers on one occasion only. The volunteers, at some definite time previously, had taken a single dose of the drug, or would do so at some definite time later. Such tests give an indication of the persistence of the drug and the times at which the parasites are most susceptible to it. The results are recorded in Tables IX and X.

We attach considerable importance to the results recorded in Table IX. The life cycle of P. falciparum is such that parasites are liberated into the blood about 144 hours after the injection of sporozoites. (Fairley 1945, 1947; Shortt, Fairley, Covell, Shute and Garnham 1949). To achieve causal prophylaxis a drug must completely prevent the release of parasites into the blood by killing all the early exo-erythrocytic forms. It has been emphasised previously (Robertson 1957) that a small dose of causal prophylactic drug given late in the early exo-erythrocytic phase may be as effective as a large dose given early in the early exo-erythrocytic phase. At the time of a once-weekly treatment early exo-erythrocytic forms may be anything from a few minutes to some 144 hours old. Effective treatment must therefore kill all stages, or the properties of the drug must be such that it persists sufficiently long for a lethal concentration to be still present at the time when the parasites become most susceptible to it. For example, from Table IX we see that with 5943 a dose such as 7.5 or 10 mgm. given at the time of biting did not always protect, but when a dose of 5 or even 1 mgm. at 120 hours after biting was given, no malaria developed. Similarly with proguanil, a smaller dose given at 96 or 120 hours after exposure is considerably more

/effective ...

TABLE X

SINGLE BITING TESTS - LAKE VICTORIA STRAIN.

Time of dosing in relation to biting. Biting time as zero.	Dose	No.of Vols.	Completely protected	Exhibiting Overt malaria
0 hours	12.5 mgm.	1	1	0
	10 mgm.	1	0	1

effective than a larger dose given at the time of exposure or 24 hours afterwards. The results we have obtained with 5943 at -24 hours and 0 hours indicate very strongly that 5943 in a dose of 20 mgm. would be effective in preventing people from becoming infected with P. falciparum if it were given once-weekly, and they give more than adequate confirmation of the results achieved in Experiments I and II.

In order to ensure that the early exo-erythrocytic forms encounter a lethal concentration of drug, it is essential that, on leaving a malarious area, drug administration does not at once cease, but that at least one further weekly dose is administered to kill any early exo-erythrocytic forms of P. falciparum arising from a bite received on the day of leaving the area. (Unless treatment is given with Primaquine, malaria caused by P. vivax may, of course, occur many months after ceasing prophylaxis with any drug.)

In contrast, from these results it can be seen why, before entering a malarious area, no prior dosing or build-up is required when a drug of the causal prophylactic type is used. In practice, however, the drug is sometimes administered first a day or two earlier, so that symptoms such as headache, nausea or tiredness, due to the upheaval of a journey, are not erroneously attributed to and associated with a drug whose first administration coincides with the day of arrival in a foreign country.

Action of 5943 on the Erythrocytic Stage :

Twenty-five out-patients and 24 in-patients at different hospitals, showing parasitaemia and fever, were given treatment with 5943. Though no patient who was dangerously ill was given the drug under test,

TABLE XI

An example of the response encountered with 5943 in an adult with nephrotic oedema requiring pyrotherapy.

	Exposed to 4 mosquito bites, Mombasa strain	Dosed with 10 mg.5943						
Day	1	15	16	17	18	19	24	29
Weight lbs.	139					133	123	128
Max. Temp.	98.6	104.5	100.2	99.4	98.4	98.4	98.4	102.4
Trophozoites cmm.	-	150	150	6	0	0	0	1200

many of the patients were suffering from acute malaria. The patients included people who had come to Nairobi from all over East Africa, so that a variety of strains were possibly involved. Most of the parasites were P. falciparum, but some patients were infected with P. vivax and P. malariae.

We did not fail to achieve clearance of trophozoites and subsidence of pyrexia within four days, usually within two, using doses such as 30 mgm. for adults and 10 mgm. for young children. The number who showed a recrudescence of parasites two or three weeks later, however, was considerable. This indicated that though the dose, as expected, was inadequate, the parasites were all susceptible to the drug, as shown on Table XI.

Toxic Action of 5943 :

Six healthy men, weighing between 60 and 70 kilos, were given 50 mgm. of 5943 once weekly for nine weeks, followed immediately by 50 mgm. twice weekly for a further five weeks. On detailed questioning, one man thought that on the higher dose he had a tendency to have looser stools but was by no means convinced of this. Towards the end of the test, one man developed a few lichenoid papules on his forearms. These disappeared shortly after the test was finished. The opportunity to readminister the drug was not possible. The remaining four men remained well throughout. In none could significant renal or haemopoietic changes be noted. One patient suffering from chronic discoid lupus erythematosis received 10 mgm. of 5943 daily for six months, and five others who had not responded to other antimalarial drugs received 20 mgm. daily for three months, omitting doses on Saturdays and Sundays. No definite side effects were noted. In most patients,

TABLE XII

Single biting tests - Malayan No.2.Strain (Resistant to Proguanil)

Time of dosing in relation to biting. Biting time as zero.	Dose	No.of vols.	Completely protected	Exhibiting overt malaria
- 168 hours	50 mg.	2	0	2
0 hours	80 mg.	2	0	2
	60 mg.	2	0	2
	40 mg.	2	0	2
	20 mg.	1	0	1

the results of treatment with 5943 were not as good as those obtained by Rogers and Finn (1954) using chloroquine, or even mepacrine, in chronic discoid lupus erythematosis and light eruption.

Resistance :

The results obtained in the early exo-erythrocytic phase, illustrated in Table XII, confirm that there is a high degree of cross-resistance with proguanil against this specially selected Malayan strain. Results obtained by Avery Jones (personal communication) in curative theraphy at Makueni indicate that, although a normal response was not obtained with 5943 against parasites showing pyremethamine resistance there, some therapeutic action was still obtained with it.

Inhibition of Sporozoite Development :

5943, as might be expected, possesses the property of proguanil for preventing the development of the oocyst in the stomach of the mosquito. One adult, showing approximately one gametocyte per field (1/12th in objective), allowed mosquitoes to feed on him, and on subsequent dissection of these mosquitoes, eight in number, all eight of the survivors were found to be heavily infected with sporozoites. Immediately after these mosquitoes had engorged on him, this volunteer was given 50 mgm. of 5943. Of the mosquitoes which fed on him the next day, all twelve failed to show sporozoites when subsequently dissected. Small batches of mosquitoes were fed on him on alternate days for a further 14 days and, on dissection at the appropriate time, none of these surviving 43 mosquitoes showed any sporozoites. Twenty-three days after dosing, however, yet another batch of mosquitoes was fed on him and, on subsequent dissection, two out of five of the surviving mosquitoes were found to show sporozoites. Throughout these 23 days there was little variation in the day-to-day gametocyte count.

SUMMARY AND CONCLUSIONS

The biguanide 5943 was tested against strains of P. falciparum and found to have considerable anti-malarial activity, with similar qualities to proguanil, but to be weight for weight more active and persistent. Against strains of P. falciparum investigated, doses of 15 mgm. once weekly would be an adequate prophylactic dose. Doses of 30 or even 50 mgm. once weekly would be well tolerated and would give a wide margin of effectiveness, considerably greater than 300 mgm. of proguanil, against any strains showing minor degrees of resistance.

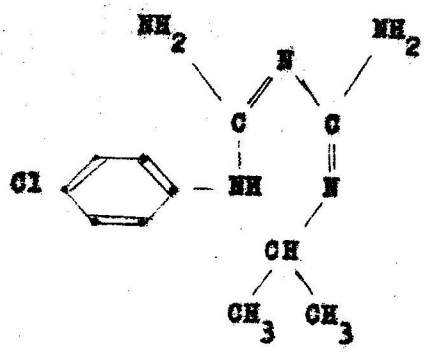
SECTION SEVEN

EXPERIMENTS WITH AN ACTIVE METABOLITE OF PROGUANIL AND AN ACTIVE METABOLITE OF 5943

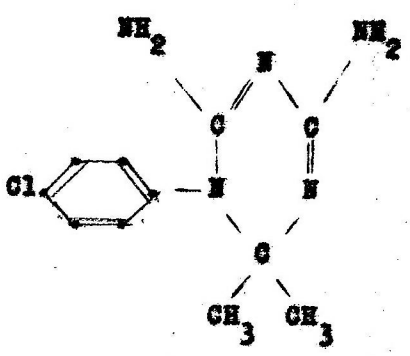
Results obtained from *in vitro* work with malarial parasites caused Hawking to postulate that proguanil was probably converted in the body to a more active substance (Hawking 1947; Hawking & Perry 1948). A metabolite of proguanil more active than the parent substance, and a similar metabolite of 5943 were later isolated an synthesised (Carrington, Crowther, Davey, Levi & Rose 1951; Crowther and Levi 1953). The relationship of the metabolites to the parent compounds is shown below. It will be seen that the metabolites are formed from the parent substances by loss of two hydrogen atoms and subsequent ring closure.

Laboratory work by Davey with *Plasmodium gallinaceum* showed that 10,580, the metabolite of proguanil, was about ten times more active than proguanil itself, and 10,732, the metabolite of 5943, about ten times more active than 10,580, and therefore about one hundred times more active than proguanil.

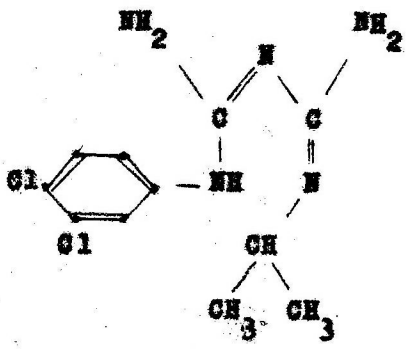
Clearly, such active compounds were worthy of trial in man, and it is the purpose of this section to describe the results achieved with them. Because 10,732 was the more active of the two new compounds, the first experiments in man were done with it.



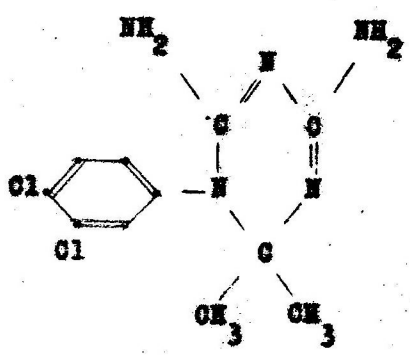
(I) **Proguanil**



(II) **10,580**



(III) **5943**



(IV) **10,732**

TABLE XIII

Summary of results of Experiments Nos.1 & 11.

Compound 10,732 - 50 mgms.weekly.

No.of volunteers	Exposure period days.	No.of doses prior to exposure	No.of doses subsequent to exposure	Total No.of doses	Strain	Approx No. of mosquitoes biting each vol.	No.dissected &%° + of batches used.	Result		
								Protected	Not protected	Day on which parasitaemia developed
10	49	0	2	9	Mombasa	125	789 90%	6	4	35 48 71 79

Compound 10,732 - 10 mgm. daily.

10	49	0	7	56	Mombasa	125	789 90%	10	0	-

Action of 10,732 on the Early Exo-erythrocytic Forms of P. falciparum :

Experiment I :

A group of ten African volunteers were exposed almost daily to infection by our standard methods. The Mombasa strain was used and the exposure period was 49 days. The dose given to each volunteer was 50 mgm. once weekly, starting on the first day of the experiment, which is the day when volunteers were first exposed to mosquitoes, and the dose was continued for two weeks after the last bite. There was then an observation period of 50 days. Each volunteer was bitten by about 125 mosquitoes. Of 789 mosquitoes from the batches used on this experiment, 711 were found to show sporozoites on dissection, an infection rate of 90 per cent.

Results : Six volunteers were fully protected from this heavy challenge; the remaining four developed parasitaemia, one man on Day 35 whilst still being bitten, another on Day 48, the last day of exposure, and the other two on the 15th and 24th days respectively after the last dose of 10,732.

Experiment II :

Another group of ten African volunteers were infected in the same way for a period of 49 days, but they received doses of 10 mgm. of 10,732 daily from the first day of infection and for seven days after the last exposure. They each received bites from approximately 125 mosquitoes, and, on dissection of a proportion (789), 90 per cent. were found to harbour sporozoites.

Results : All ten volunteers were protected throughout biting, and none developed malaria during the subsequent period of observation of at least 50 days.

These results are summarised in Table XIII.

TABLE XIV

Action of 10,732 v Mombasa Strain.

Time of dosing in relation to biting. Biting at zero hour	Dose (mg)	Nos.of vols.	Compl-etely prot-ected	Exhibiting Overt malaria
0 hours	50	2	0	2
	10	2	0	2
+ 12 hours	10	2	0	2
	5	2	0	2
24 hours	5	2	0	2
48 hours	10	2	2	0
	5	1	0	1
72 hours	5	1	0	1
96 hours	5	1	1	0
120 hours	5	1	1	0

Experiment III :

Single biting tests were carried out in our standard way with African volunteers, the biting and dosing being done at measured intervals. The results are given in Table XIV. They emphasise again the great difference in susceptibility of the exo-erythrocytic forms according to whether treatment is made at the time of biting and very shortly afterwards, or later, such as at 96 and 120 hours. In these experiments with 10,732, a single dose of 5 mgm. gave complete protection at 96 hours and at 120 hours after biting, whereas a single dose of 50 mgm. failed when given at the time of biting.

Comments on Experiments I, II and III :

I have emphasised previously that failure or success of a drug to give complete protection when it is administered at the time of biting gives a useful indication of its value for once-weekly prophylaxis against falciparum malaria. There is no doubt that 10,732 is a very active drug - witness the success of single doses of 5 mgm. at certain times in the single biting experiments (Table XIV) and the complete success of a daily dose of 10 mgm. (Experiment II) - but, probably because of failure to possess the requisite pharmacological properties, for example, persistence, it fails in doses of as much as 50 mgm. given at the time of biting (Table XIV) and therefore may fail in a similar dose given once weekly, as was demonstrated in Experiment I.

Action of 10,732 on the Erythrocytic Stage :

Thirty-six out-patients, mostly children, showing parasitaemia, but not always with symptoms attributable to it, were given 10 mgm. of 10,732, or adults 30 mgm. of 10,732, as a single curative dose.

(Symptoms of gastrointestinal disturbance occasionally followed the administration of 50 mgm., and this dose was therefore considered the maximum for routine treatment). The parasitaemia, whether falciparum, vivax or malariae, was affected by the drug and usually disappeared, except, of course, the gametocytes, within two or three days, but a recrudescence would often occur some seven or ten days later showing that, though the strains were sensitive to small quantities of the drug, it appeared to be rapidly excreted.

Although a high proportion of good results were obtained with 10 mgm. of 10,732 in children, a number of relatively poor results were also obtained, sufficient to show that a single dose treatment with 10,732 could not be recommended for use in these out-patient clinics. For example, two one-year-old children were found to be still showing large numbers of trophozoites seven days after treatment with 10 mgm. of 10,732. A third child of similar age, although becoming parasite-free on the fourth day after treatment with 10 mgm. of 10,732, returned on the twenty-first day showing a heavy parasitaemia.

Action on Gametocytes :

10,732 did not appear to have any visible effect on gametocytes, but it affects the cycle in the mosquito, probably in the same way as proguanil and 5943, by inhibiting the development of oocysts, as the following experiment shows. A seven-year-old youth, found to be infected with an unknown Kenya strain of *P. falciparum*, showed few trophozoites but the blood contained about 300 gametocytes per c.mm. Mosquitoes were fed on him and he was immediately given 10 mgm. of 10,732. Six hours later mosquitoes were fed, and again 24 hours after dosing.

TABLE XV

Action of 10,732 on Gametocytes.

10 mg. of 10,732 were given on Day 1, at 10.5 a.m. to a seven year old African male infected with an unknown Kenya strain of *Plasmodium falciparum*.

	Day 1 10 a.m.	Day 1 4 p.m.	Day 2 10 a.m
Schizonts in blood	-	-	-
Approx.no.gametocytes	300 cm.	300 c.m.	300 cm.
No.of mosquitoes which fed and available for subsequent dissection.	9	5	6
Infectivity found	9+++	5-ve	3+ 3-ve

When the mosquitoes were dissected later, nine out of nine of those which fed on him prior to dosing were found to be heavily infected with sporozoites; five out of five of those which fed six hours after the drug were found not infected, and of those which fed 24 hours after the drug had been given, three were lightly infected and three were not infected. This experiment again emphasises how relatively quickly 10,732 is either destroyed or excreted. See Table XV.

Conclusions on 10,732 :

10,732 is active against the early exo-erythrocytic and erythrocytic stages of P. falciparum in man and prevents the development of sporozoites in the mosquito. It also exhibits action against the blood forms of P. vivax and P. malariae. It does not appear to differ qualitatively from proguanil but various results suggest that the antimalarial action is less persistent, probably because it is excreted quickly. Thus :-

(i) Proguanil, given once weekly in what would appear to be, from the laboratory results against P. gallinaceum, less than equivalent doses, gives better protection. With 10,732, 50 mgm. once weekly failed to give complete protection, even though 10 mgm. at 48 hours after biting and 5 mgm. at 96 and 120 hours after biting were successful, and 10 mgm. daily was completely successful.

(ii) The experiments with gametocytes (Table XV) indicated that 10,732 disappeared from the blood relatively quickly.

(iii) The recrudescence of parasites in some of the children in whom there had been an initial rapid clearance of parasites after dosing with 10 or 20 mgm. of drug sometimes occurred rapidly.

TABLE XVI

Action of 10,580 v Mombasa Strain.

Time of dosing in relation to biting. Biting at zero hour	Dose (mg.)	Nos.of vols.	Completely protected	Exhibiting Overt malaria
0 hours	75	3	1	2
	62.5	1	0	1
	50	8	1	7
	37.5	3	1	2
	25	1	0	1
48 hours	50	3	3	0

Action of 10,580 on the Early Exo-erythrocytic Forms of P. falciparum :

Because the results achieved with 10,732 were relatively disappointing and because we thought that 10,580 would be equally disappointing, only a limited number of experiments, sufficient to confirm our pessimistic view of this compound, were done with it.

Experiment IV :

As previously, African volunteers were exposed to infection on a single occasion by several mosquitoes infected with the Mombasa strain and were given varying doses of 10,580. The results are given in Table XVI. The failure of the drug to give complete protection in volunteers who received it at the time they were bitten indicates that it would not be efficacious for once-weekly prophylaxis in reasonable doses. We think that the general poorness of the results are to be attributed, as in the case of 10,732, to the rapidity with which the drug is excreted. Better results were obtained if dosing was delayed until 48 hours after biting.

Some twelve curative tests in out-patients were made. Again, though the results were sometimes satisfactory, the proportion of patients having a rapid recrudescence after doses of 50 mgm. was unduly high, so that it could not be considered for use as a single dose treatment.

There was evidence that the Makueni strain, which was resistant to pyremethamine and somewhat resistant to proguanil, was also resistant to 10,580. For example, in the blood of one adult who had had 325 mgm. of 10,580 during one week, large numbers of trophozoites and gametocytes were present seven days after the last dose. Several mosquitoes which fed on him on that seventh day were later found to be heavily infected.

Conclusions :

10,580, as was expected, shows some cross-resistance with proguanil and pyremethamine. It appeared less active than 10,732. Evidence suggests that it is also probably excreted rapidly. In a patient infected with a pyremethamine resistant strain, who had large doses of 10,580, mosquitoes could be infected when exposed to him seven days later. High doses were also liable to cause gastrointestinal irritation.

SUMMARY

10,732 and 10,580, metabolites of 5943 and proguanil, were found to be active antimalarial compounds, similar in qualities to the parent compounds in their activity at all stages of the life cycle of P. falciparum.

They are, however, very rapidly excreted and because of this are less suitable for use as antimalarial drugs than existing drugs.

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