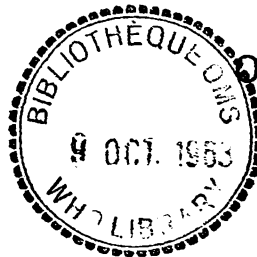


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FURTHER DEVELOPMENT OF SYNTHETIC ANTIMALARIALS<sup>1</sup>

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INTRODUCTION

The antimalarial drugs at present available are, practically always, adequate for the successful treatment of individual cases. Consequently, interest in further development of new antimalarials has gradually decreased. The initial successes achieved in malaria eradication by the use of insecticides also helped to push malaria chemotherapy into the background. The consequent stagnation is all the more understandable as research in the field has always been particularly time-consuming and costly.

In recent years however, the position has undergone a fundamental change. Today, it is generally agreed that:

- (a) the antimalarials at present available are little, if at all, suited to overcome the difficulties of mass application, and
- (b) it is imperative in malaria eradication to make use of chemotherapy as a complement to all the other control methods employed so far with such success (WHO, 1961a, 1961b, 1962a, 1962b, 1962c).

We shall consider here only a few of the problems involved, the solution of which seems particularly pressing. We shall also endeavour, in the light of a few examples, to discuss whether (and to what extent) it would be possible, by changing the test methods so far employed in the laboratory, to adopt these better to practical requirements and, to simplify and decrease the cost of further work in the field of chemotherapy.

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1. Radical treatment of *P. vivax* and *P. malariae* infections

After Sinton (Sinton & Bird, 1928; Sinton et al., 1930; League of Nations, 1937) had shown in field trials the effectiveness of pamaquine for relapsing malaria, it was found in practice that the required prolonged administration of the drug was accompanied by toxic side-effects, particularly in dark-skinned races; this militated against mass administration without constant medical control. After pamaquine, pentaquine and isopentaquine, primaquine was recommended in the United States of America and quinocide in the USSR as being more suitable. The two substances are 8-aminoquinoline derivatives and their chemical constitution is very close to that of pamaquine (WHO, 1961b, pp. 12-14). Nevertheless the advance represented by the introduction of primaquine is still not sufficient as shown by numerous individual reports in the literature and by recommendation 10.3(b) in the "Report of a Technical Meeting on Chemotherapy of Malaria" (WHO, 1961b, p. 67) which reads as follows: ". . . the Group recommends that intensive research be carried out with a view to developing an anti-relapse drug free from side-effects and radically curative at a single dose or, at most, after a three-day treatment".

Laboratory research of this nature encounters special difficulties. It is true that in his work on *P. cynomolgi* in monkeys with subsequent removal of the spleen Léon H. Schmidt (Genther et al., 1948; Schmidt et al., 1948) developed a method which makes it possible to establish in animal tests whether a chemotherapeutic agent has any anti-relapse properties; however, for the rapid screening of a large number of new compounds this expensive procedure can be used only in special circumstances. The method developed by Léon H. Schmidt continues to be of outstanding value as an intermediate control between the laboratory testing of a new compound and its clinical trial in man. The value of this test has still been enhanced by the work on the exo-erythrocytic forms of simian and human malaria carried out first by Shortt & Garnham (1948) and later by other investigators (for bibliographical references, see Eyles, 1960; Bray et al., 1963a; Bray, 1963b). To recognize any possible anti-relapse action of a compound as quickly and cheaply as possible using small laboratory animals, we have changed the usual procedure as follows: canaries are infected intramuscularly or intravenously with sporozoites of *P. cathemerium*. The treatment is started only on the fourth day after inoculation.

In this way the exo-erythrocytic (E-E) cycle is allowed to develop completely and undisturbed (Bray, 1957). An assessment of activity under these conditions gives fundamentally different results from, for example, those of the "Roehl test" (infection with parasitized blood and immediate start of treatment).

In the "E-E test" described above, substances such as quinine and mepacrine are almost without action. Pamaquine brings about a strong attenuation of the infection.

With the help of this method it was found that compounds of the pyrocatechol series have an action similar to that of pamaquine. This is all the more surprising since in the usual "Roehl test" these compounds are found to be only slightly more effective than quinine.

One of the pyrocatechol compounds known under the code name RC12 was chosen to be tested for anti-relapse action in the field, after Ramakrishnan & Basu had shown, in some random tests, that in monkeys infected with P. cynomolgi, RC12 could bring about a relapse-free cure of this infection (personal communication). In monkeys RC12 proved about 10 times less toxic than pamaquine. Field trials in P. vivax infections seem to be justified. But as the schizontocidal action of RC12 is relatively poor, radical treatment can only be tried out in combination with chloroquine.

In the laboratory the experiments in the lines described above are being continued since, according to our experience, it seems probable that this rapid and cheap screening test for substances with an anti-relapse action can be improved.

## 2. Development of new drugs

Further work in this direction was recommended by the WHO Technical Meeting (WHO 1961b). We felt it was important:

- (a) to simplify the screening methods as much as possible, to make them comparable and adapt them as far as possible to the eventual practical aims;
- (b) to search for new groups of compounds which may be expected to have a mechanism of action different from that of compounds belonging to already known groups.

In the course of our experimental work, the importance of a constant "inoculum" in achieving the aim indicated under (b) above was immediately obvious. In the screening of antimalarials too, it is necessary to observe certain conditions and principles which are regarded as a matter of course in chemotherapeutic work in other fields.

Hanson & Tatum (1952) have shown experimentally that the pamiquine effect is dependent upon the size of the "inoculum". In our own experiments we found that an "inoculum" of about 5000 parasites per canary was particularly suitable. When the number of parasites is lower (about 500 or less) or higher (50 000 or more parasites per canary) it is difficult to detect differences in the activity of the antimalarials under test. The optimum for the particular strain of parasite used must be determined by comparative experiments. These data apply to the so-called "Roehl test" (infection by parasitized blood).

As an example, we may mention that in this way and while shortening the time of treatment to only two or three days, it was possible to find highly active 6-aminoquinoline derivatives which had been synthesized by F. Schönhöfer.

Details will be reported separately at a later date. The 6-aminoquinoline compounds so far prepared, which were found in canaries superior to the 4-aminoquinoline chloroquine when given at the same dosage and under identical conditions are, however, too toxic for mammals (particularly rodents such as mice and rats) to be tested for antimalarial action in man. This work will be continued, especially as in preliminary experiments the new compounds have proved to be highly effective also in the treatment of P. cynomolgi infections in monkeys as investigated in collaboration with S. P. Ramakrishnan & P. C. Basu in Delhi (in press).

Practical conditions can be approached more closely in the laboratory by employing; in addition the "therapeutic" test which was already used by Roehl (1926). After infection with parasite-containing blood, treatment is commenced only after full development of the blood infection.

### 3. The problem of toxicity studies

In the early days of research on synthetic antimalarials (Roehl 1926) it was usual for the purpose of comparative evaluation, to determine the "chemotherapeutic index" (ratio of the maximal tolerated dose of a drug to its minimum effective dose) in the canary.

Marshall (1946) has pointed out why this concept of the "chemotherapeutic index" introduced by Ehrlich, has no practical significance for the evaluation of antimalarials. Consequently, Marshall determined the antimalaria activity in birds, using the "quinine equivalent" for comparing effectiveness and examined the toxicity of new compounds in mammals. This procedure brought about a great advance in the comparative evaluation of new antimalarials.

We have already mentioned above the importance of comparative determination of activity of a suitably chosen infective "inoculum" which is then kept constant. It is possible to increase further the rapidity and the reliability of comparative evaluation if in addition to the untreated controls, a standard preparation of known activity is included in each experimental series.

For the determination of toxicity it is well known that it is not sufficient to use only one species of mammal. It is true that, at the outset of the investigations, a certain limitation of routine tests cannot be avoided. However, in the case of new compounds, it is always necessary to individualize the pharmacological-toxicological tests appropriately in accordance with the observations made.

An example will show that such a procedure can also furnish valuable indications for further chemotherapeutic work: according to Blanchard & Schmidt (Wiselogle, 1946) the 8-aminoquinolines produce toxic effects which Findlay (1951) has classified into those affecting: (1) the haematopoietic organs and the formed elements of the blood; (2) the central nervous system; (3) the heart and circulation; to these should be added: the smooth musculature of the gut (gastro-intestinal irritation), Schmidt (Wiselogle 1946).

The cyanosis which occurs after prolonged administration of therapeutic pamaquine doses is - as we found - not caused by the formation of methaemoglobin (in these cyanosis cases, the amount of methaemoglobin is about one to two per cent.).

In such cases, there is pronounced acrocyanosis, which is circulatory in origin. Schmidt (in Wiselogle, 1946) has already pointed out that disturbances in the electrocardiogram can develop after administration of pamaquine. Further investigations along these lines have been carried out by Heimann & Shapiro (1943); Moe & Seevers (1946); Löken & Haymaker (1949); Alving et al. (1948) and many others.

We have specially investigated this important field of toxic symptoms in the laboratory in collaboration with G. Tauberger. In rabbits and, better still, cats, heart and circulatory disturbances can be detected on repeated intravenous injections of small doses of the substances under test with simultaneous recording of blood-pressure and heart-beat by Statham's method, and the electrocardiogram.

It appeared in these experiments that such disturbances occurred with the 8-aminoquinoline derivatives, pamaquine and primaquine. They are not observed with chloroquine, nor with the pyrocatechol derivative, RCl2.

However, Arora & Arora (1960) have shown in the case of amodiaquine that 4-aminoquinoline derivatives likewise can cause serious circulatory disturbances. Thus, circulatory disturbances (acrocyanosis) which are occasionally observed after the administration of antimalarial drugs occur not only with 8-aminoquinoline derivatives but may occasionally be caused by 4-aminoquinoline derivatives (cf. Smith & Schmidt, 1947).

It follows in our opinion that activity and toxicological properties are not dependent on the quinoline nucleus on the one hand and the basic side-chain on the other; it is always the particular combination of nucleus and side-chain which determines activity and toxicity.

#### 4. Causal prophylaxis and long-lasting effects

Whereas it was previously believed that all chemotherapeutic agents used for malaria control must be administered orally, in recent years the view has spread that for achieving special objectives injections can also be employed.

This applies especially to the objective of producing a "long-lasting effect" whether for causal prophylaxis or for radical treatment (WHO, 1961b). In the first instance the use of a "depot" preparation in the form of a sparingly soluble salt of a known antimalarial has been considered in this connexion. It would seem that this desideratum has been achieved by the recent development of the injectable pamoic acid salt of a triazine derivative. Other attempts in injecting sparingly soluble salts of other bases with an antimalarial effect seem to have been less successful (WHO, 1961b).

The problem of absorption-excretion is of interest in the light of our observations with Evans (Evans, 1914a, b; Schulemann 1912, 1915, 1917) on vital staining and the following consideration would also seem pertinent: compounds of small molecular size - naturally their chemical constitution is also important - are more or less rapidly absorbed from the intestinal tract after oral administration but then excreted again by the body. The relation between the time of absorption and the time of excretion is one of the factors determining the intensity and the duration of action of a drug. Beyond a certain molecular size there is no longer any absorption from the intestinal tract, so that there can be no effect. This applies, for example, to curare administered per os as well as to suramin. However, if such compounds are injected parenterally, they have an action which in the case of intravenous administration of suramin, for example, can lead to the desired long-lasting effect against trypanosomal infections.

Consequently, in addition to the attempts mentioned above to achieve a repository effect by intramuscular injection of a sparingly soluble salt of an antimalarial, we felt it desirable to investigate the possibility of producing a "suramin-like" effect by intravenous injection of compounds of high molecular weight. Technically it is easy to carry out experiments of this type in the canary. For our first trial we used intravenous injections of the otherwise very polyvalent compounds of the amidine series (stilbamidine, pentamidine, etc.). However, they had no effect on malaria infection. In the same way, a few symmetrical compounds (with two quinoline nuclei) which had been prepared by us also had no antimalarial action. The experiments are continued, in particular with a view to using asymmetrically enlarged molecules.

CONCLUSIONS

It has been our aim - by communicating some of our own experimental results - to open a discussion on the question as to whether and how the continuation of work on malaria chemotherapy, carried out on broader lines and as simply and cheaply as possible, could again be stimulated and intensified with a view to finding solutions to certain problems which, especially in regard to malaria eradication, have become of fundamental importance.

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