

Geneva, September 20th, 1938.

HEALTH ORGANISATION.

MALARIA COMMISSION.

THE ACTION OF ATEBRIN AND OF QUININE UPON THE INCIDENCE OF
THE GAMETOCYTES OF PLASMODIUM FALCIPARUM IN THE PERIPHERAL
BLOOD

by

Lieut.-Colonel J.A. Sinton, I.M.S. (retd.)
(Malaria Laboratory, Horton Hospital, Epsom).

A very large number of workers have reported that, while anti-malarial drugs belonging to the acridine series (atebrin, quinacrine and acriquine) have a definite destructive action on the asexual forms of P. vivax, P. malariae and P. falciparum, and on the sexual forms of the first two species of parasite, yet they have none on the gametocytes of the last one.* Many of these observers also state that the administration of such drugs does not prevent the formation of crescents, nor their appearance in the peripheral blood, either during or after the termination of treatment.*

Although no prolonged series of investigations were carried out at Horton to determine the influence of atebrin and of quinine upon the incidence and prevalence of crescents in the peripheral blood, yet some very interesting information can be gathered from our routine work.

(1) General Observations on the Prevalence of Crescents.

Buchanan (1902), Ross and Thomson (1910) and Thomson (1911) have pointed out that crescents usually make their first appearance in the peripheral blood about 10 days after the first asexual forms are detected. From their observations they deduced that these sexual parasites require 8-10 days to reach that stage of development at which they appear in the circulating blood. Sinton (1926) has reviewed and discussed the literature on this subject, and has produced additional evidence to support this view.

A study of the records of about 100 cases of infection with several different strains of P. falciparum (mainly Roumanian), also shows that the appearance of, or the increase in number of, gametocytes in the peripheral blood is correlated almost invariably with an increase in the number of asexual parasites about 10 days previously. This correlation appears to occur irrespective of whether the patient has been treated with either quinine or atebrin, or has remained untreated, although, as will be discussed later, treatment may affect the numerical prevalence of these forms.

* The literature on this subject has been reviewed in the IVth General Report of the Malaria Commission and its appendices (1937).

In addition, Sinton (1926) found that, in acute fresh infections with P.falciparum in India, "there seems to be a distinct correlation between the numerical prevalence of asexual parasites in the peripheral blood and the number of crescents which appear about ten days later". Kligler and Reitler (1928) in Palestine also point out that the extent of gametocyte production appears to vary directly with the intensity of infection, at least in the case of P.vivax and P.falciparum. The records of the Horton cases bear out this conclusion, in so far as our Roumanian strain of P.falciparum is concerned.

(2) The Action of Atebrin on the Prevalence of Crescents.

It is asserted by some observers that atebrin has a delayed action upon crescents, causing their disappearance 4-5 days after the cessation of treatment. Other workers suggest that this drug may have a provocative effect on such forms, in that it either stimulates the formation of crescents, or forces these out from the internal organs into the peripheral blood during the early stages of treatment.

In the past it has also been maintained by many authors that quinine treatment may act as a stimulus to the formation of crescents, a problem which has been discussed in detail by Amies (1930). This assertion appears to be based mainly upon the observation that the number of crescent carriers and the numerical prevalence of these sexual forms, are often greater about a week or so after the commencement of treatment than before it started.

In the acute cases of naturally acquired malaria studied in India by Sinton (1926), the number of crescent carriers was about 3 times as great after 4-7 days of quinine treatment as before it was given. In the present series, of 23 cases treated with doses of quinine not greater than 0.6 gm. daily before the detection of crescents in the peripheral blood, in about 26 per cent. these forms were never detected afterwards, or only in scanty numbers. Of 12 cases treated with doses of 1.0-1.3 gm. quinine, the percentage was 75, and of 19 cases treated with 1.6 - 2.0 gm. it was 94. This does not support the view that quinine acts as a stimulus to the production of crescents, because their prevalence was greater after small doses of the drug than after larger ones.

The report on atebrin published by Kingsbury (1936) also shows that the prevalence of gametocytes may be greater after treatment with this drug than before it started. Similar results were obtained in our series. Of 46 cases who received atebrin before crescents were detected in the blood, these forms appeared in about 60 per cent. of them within about 10 days after the commencement of treatment. In 54 treated with quinine under similar conditions, the percentage was 46. This might suggest that, if atebrin acts as a stimulus to gametocyte production, its action is greater than that of quinine. On the other hand, of the 26 acute attacks in which no treatment was given, crescents appeared in nearly 76 per cent. of these patients, although in many of them the prevalence of the asexual parasites never reached such a high level as that seen in the treated cases. These findings favour the view that both atebrin and quinine tend to inhibit the production of gametocytes in falciparum infections rather than acting as a stimulus to their production (vide infra).

An attack of the disease of such severity as to require treatment is usually accompanied by a coincident increase in the number of asexual parasites. If the patient survives the attack in the absence of treatment, one would expect in many instances to find gametocytes about 10 days later. It is now generally admitted that both quinine and atabrin can only destroy or prevent the development of the very earliest stages of the gametocytes, or their precursors from the asexual cycle. This being so, treatment by these drugs should not affect markedly the appearance of asexual forms within about a week or so, (i.e., at about the end of the usual course of treatment), if they had already reached the drug-resistant stage when treatment started. If the treatment were given at a very early stage, the number of gametocytes which had reached the resistant stage would be few, and, therefore, the numbers appearing later would be scanty. On the other hand, if the treatment were delayed, a greater number of young gametocytes would have arrived at the drug-resistant stage and so a greater number would appear in the peripheral blood later. This increase in crescent prevalence observed after treatment with either quinine or atabrin, is apparently only the result of the natural trophozoite-gametocyte sequence mentioned above. Just as the work of Amies (1930) goes to show that the supposed stimulating effect of quinine is fictitious, so it seems to be the case also with atabrin. The only evidence which lends any support to the view that atabrin tends to force the gametocytes out into the peripheral blood, is the early appearance after treatment of those scanty immature (?) forms described by Sinton. (1938).

The larger percentage of crescent carriers found after atabrin treatment as compared with quinine, might suggest that the former drug had a greater stimulating action than the latter. It would also be explicable if atabrin was less effective than quinine, in the doses used, against the early stages of the gametocytes or their precursors. This will be discussed later.

No evidence was found of any delayed action of atabrin. The intensity of the prevalence and the rate of disappearance of crescents after treatment, seem to depend on the intensity of the treatment and on the period of the attack at which it was instituted.

Conclusions.

The evidence available does not support the assertion that, in cases of infection with P.falciparum, either quinine or atabrin may act as a stimulus to the production of gametocytes. No delayed action of atabrin on crescents could be detected.

(3) The Comparative Effects of Atabrin and of Quinine upon the Incidence of Crescents.

Records are available of the incidence of gametocytes during and after treatment of about 100 acute attacks in early infections of P.falciparum. In these cases either quinine or atabrin was given in varying dosage.

As these drugs appear to act only upon the earliest stages of the falciparum gametocytes, our observations have been confined chiefly to the study of attacks in which treatment was given before these forms were detected in the peripheral blood.

(a) Dosage of Atebrin in relation to the Production of Crescents.

An analysis has been made of 46 instances in which this drug was given orally before the appearance of crescents in acute attacks of malaria due to P.falciparum. The results suggest the following conclusions:-

(i) A course of treatment consisting of a single dose of 0.6 gm. atebtrin followed by doses of 0.3 gm. for 5-7 days, given when parasites are not more numerous than 1 per field of the thin film, is rarely followed by the appearance of detectable crescents in the peripheral blood (6 cases).

(ii) If a single dose of 0.6 gm. atebtrin be followed by only one dose of 0.3 gm. on the next or the following day, a similar result is often obtained, but less frequently than with the longer course (5 cases). In one case in which the second dose was 0.6 gm., no gametocytes could be detected although the asexual parasites were originally 2-3 per field of the thin film before treatment started.

(iii) A single dose of 0.6 gm. given when the parasites are less than 10 per 100 fields of the thin film, is usually followed by few or no gametocytes (4 cases), but when the parasites are about 50 or more per 100 fields, these forms may be relatively numerous at a later date (5 cases).

(iv) Seven cases in which the asexual parasites varied from 1 to 180 per 100 fields were treated with 0.3 gm. atebtrin daily for 5-7 days. On only one case were scanty gametocytes found at a later date. When, however, the duration of treatment was only 2-3 days, the number of gametocytes was usually greater, more especially in those patients where the parasites numbered 1-2 per field of the thin film (5 cases).

(v) A dose of 0.3 gm. atebtrin given on two alternate days, failed to prevent the appearance of gametocytes (3 cases), although in one instance the parasites were only 1 per 20 fields.

(vi) A single dose of 0.3 - 0.4 gm. atebtrin failed to prevent the appearance of gametocytes in 9 out of 10 cases, even when the asexual parasites were less than 10 per 100 fields (2 cases).

These results suggest that, when atebtrin is given before the appearance of crescents in the peripheral blood, (a) a single dose of 0.3 gm. seems to have little influence in diminishing the number of gametocytes which appear later, (b) a dosage of 0.6 gm. atebtrin given on one day is more effective in reducing the number of crescents which appear subsequently than is the same total amount of the drug given on two or more successive days, and (c) when a course of 0.3 gm. is given for 5-7 days, the number of gametocytes carriers that develop are usually few, and this is more especially the case if the dose on the first day is 0.6 gm.

(b) Dosage of Quinine in relation to the Production of Crescents.

An analysis was made of 54 acute attacks in which quinine was given orally before the detection of crescents in the blood. In the majority of instances only a single dose was given, but in some the dose was repeated on the next or on several days.

(i) A daily dose not greater than 0.6 gm. Of 23 cases, gametocytes failed to appear in 2, were scanty or very scanty in 4, and were numerous or very numerous in 17.

(ii) A daily dose of 1 gm. Of 7 cases, in 3 no gametocytes were found afterwards, in 1 they were scanty and in the others numerous.

(iii) A daily dose of 1.3 gm. Of 5 cases, 4 showed no gametocytes and in 1 they were very scanty.

(iv) A daily dose of 1.6 gm. Of 11 cases, in 5 no gametocytes were found, and in the remainder they were only scanty or very scanty.

(v) A daily dose of 2 gm. Of 8 cases, 5 showed no sexual forms, and in 2 they were very scanty or scanty.

Gametocytes appeared in nearly every case treated with a daily dose of quinine not exceeding 0.6 gm., irrespective of whether the asexual forms were scanty or numerous when the treatment started. With doses of 1 gm. and over, sexual forms were more commonly found when the asexual forms had been abundant at the commencement of treatment.

These results appear to indicate that a single dose of quinine not greater than 0.6 gm. daily, has little effect in preventing the appearance, or diminishing the numerical prevalence, of gametocytes at a later date. The value of this drug as an "eventual gametocytocide" seems to increase with an increase in the amount of the daily dosage over 0.6 gm. Data collected by Sinton (1926) also suggests that treatment by small doses of this drug is followed by more gametocyte carriers than is the use of larger doses.

A study of our results also suggests that, as in the case of atabrin, a large dosage of quinine given during one day is better than the same total amount spread over two or more days. This is probably due to the higher concentration of the drug obtained temporarily in the body. At the same time there is a greater chance of catching the pre-gametocytes a day earlier, i.e., before many of them reach the drug-resistant stage.

(c) Discussion.

With quinine and atabrin, treatment given in appropriate dosage when the number of asexual parasites are still scanty, was followed by fewer crescent carriers and by a lower gametocyte intensity in these cases than when treatment was commenced at a time when the asexual forms were numerous.

There is almost certainly in the early stages of infection, a close relationship between the number of asexual

forms and the number of gametocytes which appear later. When the asexual parasites are less than 10 per 100 fields of the thin film, it usually means that the acute attack has not been going on very long and so few of the young gametocytes have had time to reach the stage of drug resistance. In this case early treatment should prevent the further development of most of these forms. At the same time, if the treatment be sufficient to cause a great decrease in the number of asexual forms the number of pre-gametocytes produced will also be diminished.

When the treatment was given in our cases at times when the asexual parasites were numerous, the attack had usually been in progress for some time. Under these conditions, many pre-gametocytes must have been formed and many of them must have reached the drug-resistant stage of growth. One would expect, therefore, that such cases would show, as they did, gametocytes more frequently and in greater numbers in the peripheral blood in spite of treatment.

These findings emphasise the importance of early treatment in falciparum infections as a means of reducing their potentialities as gametocyte producers.

It is considered by some workers that a dose of 1.0-1.3 gm. of quinine is equivalent in its schizontocidal power to 0.3 gm of atebtrin. When a comparison was made between 19 of our cases treated with 0.3 gm. atebtrin and 12 cases given 1.0-1.3 gm. quinine, it was found that in the former series about 53 per cent. of the cases showed few or no gametocytes at a later date as compared with about 75 per cent in the quinine series. In another lot of 15 cases given 0.6 gm. atebtrin, the results were about 60 per cent., as compared with nearly 95 per cent. of 19 cases given 1.6-2.0 gm. quinine. These findings suggest that, in so far as their power to inhibit the production of gametocytes is concerned, a dose of 0.3 gm. or 0.6 gm. of atebtrin is less effective than a dose of 1.0 gm or 2.0 gm of quinine respectively, at least with the Roumanian strain of P.falciparum studied by us. The number of cases reported are too few, however, upon which to make any dogmatic statement, but the results are suggestive.

A relatively large dosage of atebtrin (0.6 gm.) or of quinine (1.6-2.0 gm.) given on one day, appeared to be more effective in reducing the number of crescent carriers found later and the intensity of their gametocyte infections, than did the same total amount of the drugs given during a period of 2 or more days. These findings suggest that, in the treatment of malignant tertian malaria, it would be advantageous to give at least one large dose of either atebtrin or quinine to patients as early as possible in the infection. By this means one would probably diminish the likelihood of the appearance of gametocytes in the blood, and, if they should appear, their numbers would probably be much less numerous than after the use of smaller daily doses. This would help to reduce the risks of infection being acquired by mosquitoes.

(4) Conclusions.

Under the conditions of our experiments with falciparum infections, the evidence obtained suggests the following tentative conclusions:-

(a) An increase in the number of gametocytes is almost invariably associated with an increase in the number of trophozoites about 10 days previously.

(b) The numerical prevalence of gametocytes in the early stages of fresh infections, seems to vary directly with the previous prevalence of asexual parasites, unless modified by treatment.

(c) No definite evidence could be obtained that either atebrin or quinine has any very marked influence in stimulating the appearance of gametocytes in the peripheral blood. The post-therapeutic increases recorded appear to be simply the expression of the normal trophozoite-gametocyte sequence.

(d) When a single daily dose of 0.3 grm. and 0.6 grm. of atebrin was compared with doses of 1.0-1.3 grm. and 1.6-2.0 grm. of quinine respectively, the former drug appeared to be less effective in reducing the number of gametocytes which appeared during and shortly after treatment.

(e) Fewer gametocytes appear in the peripheral blood when a large dosage of atebrin (0.6 grm.) or of quinine (2.0 grm.) is given on one day than when the same total amount is given during 2 or more days.

(f) The earlier intensive treatment is started in a falciparum infection, the fewer will be the gametocytes produced.

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