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Malaria Commission.

The Medical Director has the honour to forward herewith a note on "SOME METHODS BY WHICH THERAPEUTIC PROBLEMS OF MALARIA ARE BEING STUDIED" (by Colonel S.P. James of the Ministry of Health, London).

This note contains a description of the methods to be utilised for carrying out experiments with the various anti-malaria drugs and may furnish guiding lines to the experts who have kindly accepted to assess the therapeutic value of Totaquina in human malaria. If these methods are being generally utilised the results obtained would be strictly comparable.

A. Biological tests with laboratory animals.

1. Therapeutic tests on the parasites of avian malaria - type "Pratesoma".

These tests, which we owe primarily to discoveries by Copanaris, the brothers Sergent, Giemsa and Marks, were established as a routine laboratory practice by Roehl who adopted the procedure of administering the drugs to the birds through an oesophageal tube passed into the stomach.

Method. Canaries are infected with a known species of bird malaria (usually Plasmodium relictum Grassi and Feletti) by direct blood inoculation intramuscularly or intraperitoneally. Professor Giemsa reports that intramuscular inoculation is best as it causes the parasites to appear in the peripheral blood almost invariably on the 5th to the 7th day after infection, while the interval is longer and not so regular when the blood is injected intraperitoneally. Professor Sergent, on the other hand, gives preference to the intraperitoneal method. Whichever

method is used, it must be adopted for all the birds (treated and untreated) used in the test experiments. The blood used for infecting the birds is taken when the parasites have reached their greatest number. It may be injected whole or may be diluted with physiological solution. Four hours after infection, treatment with the drug to be tested is begun and is continued daily for six days. The drug is administered through an oesophageal tube passed into the stomach, the usual dose being 1 c.c. of a solution of known strength for each 20 grammes body-weight of the bird. Giemsa, however, recommends that the strength should be calculated so that the quantity of liquid for a dose should not exceed 0.5 cc. With this small quantity of liquid there is less likelihood that some will be rejected by the bird. For the same reason Sergent recommends the subcutaneous method when the drug allows it.

After ascertaining the maximum strength of the solution which the bird is able to tolerate in the dose given, a series of experiments is made to ascertain whether this strength of the drug causes delay in the appearance of parasites in the peripheral blood, and, if so, what further dilutions also cause a distinct delaying effect. For example, when the drug to be administered is hydrochlorate of quinine, it is found that canaries can tolerate 1 c.c. per 20 grammes body-weight of a 1 in 200 solution and that dilutions up to 1 in 300 have a distinct effect in delaying the appearance of parasites in the blood. The range of action in quinine (therapeutic co-efficient) is therefore 1:4. The period of delay is ascertained by examining thin blood films every day, the first day on which parasites can be found being compared with the first day on which they can be found in untreated control birds. In the control birds the parasites generally appear on the fifth day after infection. A drug is not considered as having a distinct therapeutic effect unless the delay in the appearance of parasites in the blood of treated birds is at least to the tenth day.

The birds used should, whenever possible, be obtained from a country where natural infection with avian malaria parasites does not occur. When it cannot be guaranteed that the birds are free from previous infection, each bird should be examined prior to its inclusion in the series. It may be necessary to inoculate a specimen of blood into another bird to prove freedom from infection.

The test is used chiefly (1) for the preliminary purpose of selecting from the numerous preparations which are submitted for examination as to their possible antimalarial action those which give an indication that they may be worthy of further study. The synthetic preparation now called plasmoquine is an example of an antimalarial remedy found by means of the test. Its effect as compared with quinine was found by Roehl to be as follows:

	Strength of maximum tolerated dose.	Range of effective action.	Therapeutic coefficient.
Quinine	1 in 200	1 in 200 to 1 in 800	1 : 4
Plasmoquine	1 in 1500	1 in 1500 to 1 in 50,000	1 : 30

The chief limitation of the test for the purpose of discovering new remedies is that a drug which is found to be effective against the parasites of bird malaria may be found to have little or no effect against those of human malaria and vice versa. This is why successful results obtained with particular preparations in these tests are not now regarded as indicating more than that the particular preparation may be worthy of further study. (2) The test is also used for comparing the relative antimalarial efficacy of two preparations containing the same active principles but in different quantities. Using the test for this purpose

Professor Giemsa has recently shown that mixtures of the total alkaloids present in cinchona bark are effect in proportion to the amount of quinine which they contain.

2. Therapeutic tests on the parasites of avian malaria type "Halteridium".

As long ago as 1906 the brothers Sargent showed that quinine has no curative action against parasites of the type "halteridium" (Haemoproteus columbae) which occur naturally in pigeons and other birds. Since 1908 it has been the practice to try the effect of other drugs on these parasites as well as on those of the type "proteosoma". The species of parasite commonly worked with is Haemoproteus orizivorae which occurs as a natural infection in rice finches from Java and some other Eastern countries. It is a parasite of which only the gametocytes (male and female) can be found in the peripheral blood, the vegetative (non-sexual) cycle being passed in the endothelial cells of the lungs, the liver and other internal organs. Thus the object of therapeutic tests with this parasite is chiefly to ascertain whether the drug to be tested has a gametocidal action. For this purpose one selects two or three naturally infected finches and after having made a daily count of the parasites during a short period (four or five days) one treats the bird with the drug in the same way as in trials on "proteosoma" parasites of canaries. The effect of the drug is observed by noting whether the parasites disappear or are greatly diminished in numbers as a result of the treatment. Results are usually stated in terms of the optimum curative dose expressed as a fraction of the maximum tolerated dose (the dose which is just inferior to the dose which kills the bird).

Example: $\frac{\text{Maximum tolerated dose } 0.00025 \text{ g.}}{\text{Optimum curative dose } 0.000025 \text{ g.}} = 10$ (therapeutic coeff.)

3. Therapeutic tests on the malaria parasites of monkeys.

It has recently been found that several varieties of malaria parasite which occur naturally in monkeys in the Far East and in Africa can be utilised for experimentally controlled therapeutic tests of antimalarial remedies. In particular it has been found that a species of malaria parasite occurring naturally in monkeys of the genus Cercopithecus, when transferred by blood inoculation to Macacus rhesus or sinicus causes an acute infection which, when untreated invariably ends fatally, but when treated with quinine is cured or can be maintained at a low parasite level for months. In India, the Malay States and England a beginning has been made in the application of this finding to the practical purposes of therapeutic tests, and already some unpublished results of a few carefully controlled trials of the synthetic preparation atebirin in comparison with quinine have been reported to the Commission. The ability to work on the subject with monkeys as well as with birds is a noteworthy addition to the means at disposal for evaluating the relative efficacy of antimalarial remedies.

B. Biological tests on human malaria.

4. Therapeutic tests on human malaria induced intentionally in the practice of malariatherapy.

Since the introduction of the practice of malariatherapy it has become possible to study the therapeutics of the disease under controlled conditions and to devise some useful tests for

ascertaining the efficacy of preparations for prophylactic and curative purposes. It is to be understood, of course, that in all therapeutic tests on induced malaria every endeavour should be made to imitate natural conditions as closely as possible and that an essential condition is that the patients must be infected in the natural way by the bites of infected mosquitoes and not by the direct inoculation of malarial blood.

Test to ascertain if a drug is a true causal prophylactic.

The following examples (which also serve to prove that quinine is not a true causal prophylactic) illustrate the mode of conducting this form of test:

Case	How infected	Quinine given	Result		
			Fever	Parasites	Course of attack.
I	Bitten by mosquitoes, 10 a.m., 8th August, 1927 (Moderate infection.)	:10 grains 10 minutes before biting. :10 grains 30 minutes after biting. :10 grains daily (in two doses) during the next 8 days. :(Total, 100 grains)	Fever began on 9th day after infection, which was the last day on which quinine was given.	Parasites first found on 17th day after infection.	Patient had 11 paroxysms of quotidian fever. Relapsed 11 weeks after recovery.
II	Bitten by mosquitoes, 14th Sept., 1927 (Heavy infection.)	:10 grains (in two doses) daily for the 3 days prior to infection. :20 grains on the day of infection. :10 grains (in two doses) daily for the next 10 days. :(Total, 150 grains)	Fever began on 9th day after infection, which was 1 day before the end of quinine administration.	Parasites first found on 18th day after infection.	Patient allowed 7 paroxysms tertian fever. Relapsed 8 weeks after recovery.
III	Bitten by mosquitoes, 1st Nov., 1927 (Heavy infection)	:10 grains 10 minutes before biting. :10 grains 30 minutes after biting. :10 grains daily (in two doses) for the next 12 days. :(Total, 140 grains)	Fever began on 19th day after infection, which was the 7th day after quinine was stopped	Parasites first found on 26th day after infection	Patient allowed 10 paroxysms tertian fever. No relapse.

Test to ascertain the efficacy of a drug as a clinical prophylactic.

Graph No. 1 is a record of a test conducted with the object of ascertaining whether a daily dose of 5 grains (30 centigrammes) of quinine suffices to suppress the appearance of clinical symptoms and of parasites in a person who is repeatedly bitten by infective mosquitoes. The period of the trial was five weeks during which the person was infected and reinfected three times in each week.

It will be seen that while the person was actually taking the daily dose (15 Aug. to 20 Sept.) he had only one attack of fever which was suppressed within a few days, but that shortly after the cessation of the daily dose there was a severe attack. The conclusion is that a daily dose of 30 centigrammes of quinine has a good action as a clinical prophylactic but is not entirely effective for that purpose; also that its action ceases as soon as the daily doses are discontinued.

Test to ascertain the efficacy of a drug for treatment of the attack.

For this purpose it is usual to endeavour to compare the action of the drug to be tested with the action which it is known would be produced by quinine in the same circumstances. A trustworthy test with this object can only be made upon a series of patients in a primary attack and only upon those in whom the primary attack has lasted in an untreated condition the same length

length of time. These requirements follow from the knowledge

(1) that the effect of quinine is quite different in the early days of an attack, in the middle of an attack and towards its termination;

(2) that the effect of quinine is quite different in a person who has already acquired some "tolerance" from its action in a person who has none. Useful comparative tests cannot be made upon relapses because a relapse in a person who xxxxxxxxxxxxxxxxxxxx possesses a good amount of tolerance often disappears without any treatment while a relapse in a person who possesses no tolerance may last a long time and be quite severe. Other requirements for useful tests are;

(1) that all the persons in the series must be infected by the bites of approximately the same number of mosquitoes infected with the same strain of the specific parasite on which the tests are being made; (see graphs).

(2) that regular daily counts of the parasites present in the peripheral blood shall be made;

(3) that the therapeutic comparative tests are made with the smallest effective doses of the drugs used.

Method. In a large series of cases bitten by mosquitoes infected with the same strain of P. vivax, ascertain what is the effect on the fever and on the parasites of giving between the seventh and tenth day of primary attack a single dose of 0.3 gramme quinine. In nearly all the cases the result will be a temporary cessation of the febrile paroxysms and temporary disappearance of the parasites from the peripheral blood. Usually within 24 hours after giving the dose, the number of parasites falls to about half the number previously found and after 72 hours blood examination gives a negative result. Absence of fever and of parasites lasts for a period which is approximately equal to the incubation period of

the primary attack. This "period of remission" is then followed by a return of fever and parasites.

Now try the same plan with a single dose of the drug to be tested starting with a dose which, it is thought, might produce the same effect as is known to be produced by a single dose of 0.3 gramme quinine. If the single dose selected has no marked action try double the amount in the next case and proceed in this way until it has been ascertained what single dose of the drug is required to produce as good an effect as is produced by a single dose of 0.3 gramme quinine.

Example: In a series of 50 cases of benign tertian malaria (P. vivax) intentionally induced by the bites of infected mosquitoes, it was found that the administration of one dose of 0.3 gramme quinine bihydrochloride between the seventh and tenth day of the primary attack brought about in 90% of the cases a temporary correction of the febrile paroxysms and temporary disappearance of parasites from the peripheral blood. A similar series of cases infected with the same strain of P. vivax by the bites of the same batch of mosquitoes was then used for a similar test with various reputed antimalarial remedies. Two charts () are reproduced as examples showing that the particular drug tested was ineffective in a dose of 0.3 gramme but quite effective in a dose of 0.6 gramme.

5. Therapeutic tests on human malaria contracted in the field.

In general, the only test which can be conducted on patients who contract their infection in the field is a test of the immediate therapeutic value of a short course of the drug to be tested in comparison with the immediate therapeutic value of the same course with quinine.

The following is the method suggested by the Commission for the trials of Totaquina:

Procedure:

a) If possible weigh the patient and administer the drug by the mouth daily in the proportion of 0.60 gm. for benign tertian, 1.20 gm. for malignant tertian or quartan per 70 kg. body-weight. The medicament should be given in tablet form.

b) Only patients with acute clinical forms of malaria and schizonts circulating in the blood to be treated. The dose should be given under the direct supervision of the doctor.

c) Continue the treatment of cases by the drug under test or by quinine controlled by daily blood examination, until five doses have been taken (5 days).

d) Note carefully any secondary effects e.g. nausea, albuminuria, etc.

e) Comparison will be made of the lapse of time required for the complete disappearance of the schizonts from the blood according to a thorough study of the preparations. The blood examinations should be carried out by the same person using the same technique throughout the whole duration of the experiment.

f) It is desirable to make the experiment on groups of 50 patients chosen without discrimination for each drug.

g) The description of the dosage, symptoms and results must be written on the special forms provided by the Health Organisation of the League of Nations.

h) The nature of the attack should be determined as accurately as possible. It is desirable in particular to know:

- 1) if it is a primary attack,
- 2) a relapse
- 3) a reinfection.

The description of previous anti-malarial treatment should be given on the form.

