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HEALTH ORGANISATION.

MALARIA COMMISSION.

The Secretary of the Malaria Commission has the honour to communicate herewith a Note by Dr. G. T. BADENSKI of Bucarest describing the technique employed in malariatherapy in the Centres at Rome and Horton (England).

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In the following note a similar plan is adopted in describing the technique employed in Rome and at Horton. The following points will be dealt with:

- 1) Organisation of the malariatherapy services;
- 2) Strains employed;
- 3) Inoculations with virulent blood;
- 4) Inoculations by mosquito bites infected experimentally;
- 5) Suitable cases for malariatherapy and utilisation of this method of treatment for malaria cases of long standing;
- 6) Supervision of patients after clinical cure has been effected and they have left the hospital.

MALARIATHERAPY CENTRE IN ROME.

(a) Organisation of the Service.

The Malariatherapy Centre in Rome is housed in the "Santa Maria della Pietà" Hospital for Mental Diseases at Monte Mario, a suburb of Rome. It consists of a laboratory directed by a doctor who is also a malariologist (Chief of the Laboratory, Dr. E. Mosna). The work relating to malariology is carried out under the direction and supervision of the Section of Experimental Malariology (Director, Professor Missiroli) of the Italian Public Health Institute, on the lines suggested by the senior physicians of the various hospital services as regards the selection of patients, the time at which they are to be infected with malaria, etc.

The laboratory is housed in a special building which also contains the post mortem room and is situated some distance from the wards. The laboratory consists of only one room which is however spacious and can easily contain both the equipment needed for breeding and supervising the infected anopheles, as well as tables for examining blood and anopheles.

All openings are screened.

Patients are never inoculated without the consent of their family and the nearest relative must sign a form beforehand giving this consent.

The laboratory receives daily from the various hospital services lists of the patients to be inoculated. The Chief of the Laboratory may, according to circumstances, postpone inoculation for a few days. The laboratory performs inoculations in the hospital and, at the express request of the various doctors in charge of the cases, in other hospitals at Rome as well as on a large number of patients in private hospitals.

(b) Strains employed.

The strains employed for treatment are always strains of benign tertian and quartan. The hospital doctors refuse to allow their patients to run the risk of blackwater fever as a result of inoculation with malignant tertian.

At present, three B.T. strains are employed: two native strains and one Madagascar strain. A native quartan strain is also used.

The number of strains used has varied from year to year with the results of experience, of the small number of patients to be inoculated, or the high percentage enjoying unsuspected immunity.

The purity of the strains is ensured:

- (1) By always inoculating, if possible, several patients at a time with the same strain;
- (2) By inoculating with B.T. a patient who has formerly suffered from quartan malaria and vice versa;
- (3) By using anopheles for the transmission of the disease.

(c) Inoculation with virulent blood.

Inoculation with virulent blood is ordinarily used in the case of quartan. It is also used for B.T. in the following cases:

- (1) In the case of an urgent inoculation;
- (2) When the batches of infected anopheles are exhausted;
- (3) For certain experiments, such as the comparison of the degree of virulence of the same strain when introduced by the injection of virulent blood or by the bites of mosquitoes.

The quantity used, 10 cc., is given by intra-muscular injection, preferably into the buttocks. The blood is spread in a ring around the point of inoculation by withdrawing and re-inserting the needle several times, care being taken not to withdraw the needle completely from the skin. Inoculation is followed by light massage for two to three minutes.

(d) Inoculation by mosquito bites.

This method is only employed for B.T. It possesses the following advantages over inoculations with virulent blood:

- (1) It often gives positive results with the same strain when inoculation with virulent blood has yielded or would yield only a negative result;
- (2) It prevents the possible transmission of syphilitic infection;
- (3) It produces an infection comparable in every respect to natural infection - a great advantage from the point of view of malariological research;
- (4) It thus affords comparable results regarding the action of various anti-malarial drugs, etc.;
- (5) It enables the purity of the strains employed to be easily maintained.

The anopheles employed for purposes of infection come either from regions where they are present in large numbers but which are not malarial, or are bred in the laboratory. For preference they are always caught in places infested by one of the varieties of A. Maculipennis. For breeding purposes, the various varieties of this anopheles are also chosen; but in the former case the varieties must necessarily be mixed.

Anopheles bred in the laboratory are used almost exclusively all the year round. Only during November, when breeding is impossible, are infected batches used which have been caught in the open.

The mortality rate among anopheles caught in the open is always very high - 70 or 80 per cent or even higher. On the other hand, this rate is in the neighbourhood of 40 per cent in the case of batches bred in the laboratory.

For breeding purposes a large thermostat is used in which the water is kept at a constant temperature of 27°C. This thermostat, which has a glass cover, is placed near the window with the most sunlight. It is large enough to contain 12 big earthenware dishes with a top diameter of about 35 cm. These dishes are filled with spring water and used for hatching the eggs. The larvae are fed artificially until they change into pupae.

During the hot weather, when the internal day temperature of the laboratory often exceeds the 27°C. on the thermostat, three large rectangular dishes with glass sides are used for breeding; the eggs thus hatch out at the laboratory temperature.

The pupae are collected in small glass jars which are placed in gauze cages.

The anopheles thus obtained are transferred in batches of 30 to small cages, also of gauze, in which they can readily feed on guinea-pigs or rabbits. The cages are enclosed in a thermostat kept at 25°, the inner walls of which are hung with wet cloths. The same cages are employed for infecting batches of mosquitoes and patients.

Before the anopheles are infected the blood of the donor is tested. When in the fresh state it should show the exflagellation of the male gametocytes. After the blood meal the anopheles which have not bitten are removed and the cages containing the anopheles gorged with blood are put back into the thermostat until sporozoites appear in the salivary glands. During this time the anopheles are fed every three days on the same patient if the latter has not taken any medicament.

The percentage of infection of the batches is ascertained by the daily dissection of the dead anopheles, coming from outside as well as from among the laboratory-bred batches.

Once the sporozoites have appeared in the glands the cages are placed in an ice chest at  $\pm 4^{\circ}\text{C}$ ., after one day's acclimatisation to the surrounding temperature.

The infected batches are kept in the ice chest for two months. At the end of this period they are no longer capable of transmitting infection.

In the case of inoculations the batches are placed in the thermostat at  $25^{\circ}\text{C}$ . for 16 to 18 hours before the patient is infected.

A careful record is kept in the laboratory of the various dates relating to breeding and the infection of the different batches.

#### (e) Patients.

The selection of patients to be infected rests with the doctors of the various services.

Infection with malaria is often asked for even in the case of diseases other than progressive general paralysis, and the maintenance of the strains is usually a comparatively easy matter.

The main difficulties which always have to be overcome in a malarial district are as follows: the presence either of patients who have formerly had malaria and are more or less immune to any future attempt at infection, or of persons with a latent infection which can easily be made active by further inoculation. Efforts are made to remedy these drawbacks: 1) by making careful enquiries as to the patient's medical history with reference to malaria; 2) by using a suitable strain, i.e. a strain of foreign origin - for instance a quartan in the case of a patient who has previously had spontaneous attacks of B.T.; 3) by inoculating the patient whenever possible through the bites of anopheles.

The treatment by infection is decided upon in consultation with the doctor in charge of the case as regards the time of inoculation which, however, may be anticipated if necessary. Directions regarding doses and the choice of drugs are given by the Director of the Section of Experimental Malaria.

The blood is tested daily and the results are recorded from the day of infection until after the third or fourth negative result.

The temperature is taken every 4 hours during attacks. All temperature charts are kept by the laboratory.

After the parasites have disappeared and if the general condition of the patient is satisfactory, he is only kept under clinical observation.

The doctor in charge of the case reports recrudescences and relapses to the chief of the laboratory.

f) Supervision of patients after they have left the hospital:

After he has left the hospital, a report on the patient is communicated to the doctor in charge of the case or the medical officer of the place to which he proceeds, and his case is followed up by correspondence.

## II. MALARIATHERAPY CENTRE AT HORTON.

a) Organisation of the service :

The Malariatherapy Centre at Horton is housed in the hospital for mental diseases of that name, near Epsom, a town in Surrey about 20 miles from London.

The Centre is under the direction of Colonel James, adviser on infectious and tropical diseases to the British Ministry of Health; Mr. P.G. Shute is in charge of the laboratory. Dr. Nicol, director of the hospital, assisted by Dr. Hutton is responsible for the clinical supervision of the patients.

The Centre is housed in a special building and has three wards: one for women, one for men and a third kept in reserve. Two rooms are devoted to the breeding of anopheles and two to the laboratory; there are also the necessary offices.

Patients' wards and the breeding rooms are screened.

The patients are sent to the Centre and after diagnosis are transferred to the observation ward. Inoculations with malaria are performed only in cases of progressive general paralysis. As in Italy, the patient's family must give its consent beforehand. As soon as treatment has been completed the patients are transferred to one of the various hospital departments.

The Malariatherapy Centre at Horton is the only one of its kind in England. If malariatherapy is carried out in other hospitals, the latter work in conjunction with various parasitological or bacteriological laboratories in connection with the universities or merely preserve the strains used (for the most part sent from Horton) and only inoculate virulent blood.

For this reason the Horton Centre is often called upon to perform inoculations not only in the hospitals in London and the vicinity, but in all parts of the country and in the Irish Free State.

b) Strains employed:

The three strains at present employed which have been used for a long time past are: one strain of B.T. and one strain of M.T. of Roumanian origin; one strain of quartan of Dutch origin. The strains were originally selected for their infective power, and no deaths after inoculation with malaria or cases of blackwater fever as the result of inoculation with M.T. have occurred.

The purity of the strains can be ensured without much difficulty since England is a non-malarial country (50 cases of spontaneous malaria in 1919 is the largest number recorded from that date to this). Even if the patients to be inoculated with malaria often include a percentage of old malaria cases, that percentage is fairly low. As will be seen later, the use of anopheles considerably facilitates the task.

(c) Inoculation with virulent blood.

Sub-cutaneous injection, which is the rule for quartan, is no longer used in the case of the other strains.

Inoculations with virulent blood are only performed if there are no batches of infected anopheles available. This has not happened for the last two years.

At the present time inoculations with virulent blood are confined to distant hospitals to which the defibrinated blood is sent by post.

The quantity of blood used is 5 cc. This dose is injected subcutaneously into the sub-scapular region, in the form of a ring round the point at which the needle is inserted. Inoculation is followed by light massage of the region.

(d) Inoculation through the bites of anopheles.

Inoculation by mosquito bites is always performed with A. maculipennis, atroparvus variety. This variety, which is of native origin, has been bred in the laboratory since August 1933 and now comprises a large number of successive and uninterrupted generations.

The Centre possesses two rooms specially fitted up for breeding purposes. They are kept at a constant temperature of 23.9°C. (75°F.), the moisture in the atmosphere being about 80° measured by the hygrometer.

The first of these rooms is used exclusively for breeding larvae. Wide earthenware dishes are placed all round the room. Tufts of freshly-cut grass are placed round the edges of the dishes and they are filled with rainwater so that the roots of the tufts and the small quantity of earth adhering to the roots are covered. Egg-laying then follows. The larvae hatched do not receive any external nourishment. The surrounding temperature permits of the development of a very abundant bacterial and infusorial flora on which the larvae feed. When this flora is sufficiently abundant to form a film, the water in the basins is aerated with a rotary pump. When the larvae reach their third stage, their voracity prevents the formation of a film and aeration becomes unnecessary.

The pupae are collected in small glass jars which are placed in gauze cages where the larvae hatch out.

The cages used for this purpose, and those containing the various batches of infected anopheles, are placed in the second breeding room.

Before the anopheles are infected, the blood of the donor is always tested for the purpose of ascertaining the percentage of male gametocytes which have reached maturity. Their maturity (ex-flagellation) is ascertained by means of smears, in a damp room, by Shute's method.

The batches are not regarded as infected if the percentage falls below a minimum of 180 - 280 ripe gametocytes per cmm., and if it reaches that figure the blood meal is repeated two or three times.

By this means a daily infection, varying between 80 and 100% for B.T. and of about 70% for M.T. is obtained.

The percentage of infection among the anopheles is ascertained by daily dissections.

As soon as the sporozoites are found in the salivary glands - (in the case of batches infected with B.T.) - the anopheles are kept in the ice chest at a temperature of  $\pm 10^{\circ}\text{F} = - 12.2^{\circ}\text{C}$ . The batches infected with M.T. cannot be kept in this way as the mosquitoes are unable to stand such a low temperature when they have been infected with malignant tertian and consequently die. These batches are kept at a temperature of  $- 9.40$  to  $- 3.85^{\circ}\text{C}$  ( $15$  to  $25^{\circ}\text{F}$ ).

Heavily infected batches i.e. those with a large number of sporozoites in their glands, are kept in the ice chest, that is to say, without the necessity for a meal every two or three days, and hence without any loss of sporozoites. As many as 30 patients can be infected by this means over a period of about three or four months.

When describing the methods used at the Rome Centre, I mentioned the advantages of inoculation by mosquito bites, I also stated that for more than two years past, the Horton Centre had used anopheles exclusively for inoculations in cases of B.T. as well as M.T.

I should add that this method is being increasingly used for inoculations performed in the neighbouring hospitals and in many cases in distant hospitals.

(e) Patients.

Although England is not a malarial country, a large number of Englishmen reside or travel in tropical or other regions infested with malaria. The enquiries made from each patient enable the doctor to know whether there is any possibility of acquired immunity or relapses of latent infections. It should be noted that this applies chiefly to men.

Once a certain strain has been used for infecting a patient with malaria, the blood is tested daily until three successive negative results have been obtained. The temperature is taken five times each day at four hourly intervals.

At the onset of an attack the temperature is taken every fifteen minutes; when it falls it is taken every four hours as previously. A record of the patient's temperature is kept during the whole of the time he remains in the ward.

As soon as his general condition allows him to be moved, the patient is transferred to one of the hospital departments, where he is only kept under clinical supervision. As soon as a relapse occurs, the patient is moved to the Centre where the observations are resumed as before. The Centre keeps daily records of the results of the blood tests, the anti-malarial treatment employed, the result of inoculations and the number of batches of anopheles.

(f) Supervision of patients after they have left the hospital.

Once the patients have left the hospital, supervision is exercised in two forms: Patients, living near come back every two months for a complete medical examination; In the case of a relapse, patients are asked to apply to the hospital for quinine, which is supplied gratuitously.

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