Editorial.

MALARIA.

In an Editorial on the State of the Public Health we referred to some of the most recent work on malaria outlined in Sir George Newman's Report.

The new knowledge has been largely obtained from the study of malaria purposely induced in patients suffering from general paralysis. Wagner Jauregg began the treatment of general paralysis by artificially infecting the patient with malaria parasites; this led Colonel S. P. James to introduce natural inoculation through the mosquito, and so to formulate a method by which clinical and therapeutic tests could be carried out on the human subject in Europe.

Colonel James carried out his investigations at the Horton Mental Hospital. His practical object was to induce a pure infection of benign tertian malaria in patients to be treated. The strain of *Plasmodium vivax* used in his early work was obtained from India; this strain was lost during an unavoidable interruption of the work and a strain obtained from Madagascar was then employed. The mosquitoes (female *maculipennis*) were collected in the adult stage in a country district where malaria does not occur. Between 1923 and 1926, about 3,200 mosquitoes were used in 22 batches, but only 715 lived long enough to be available for infecting patients. During the period 221 patients were subjected to bites and 169 developed benign tertian malaria within the usual incubation period of the disease. In 1926, Colonel James furnished a report on the first results of laboratory work on Malaria in England to the League of Nations.

Discussing the factors relating to the transmission of malaria from man to the mosquito, he states that a small number of patients suffering from malaria are infective to anopheles, a very small minority being "good infectors." In a first attack of malaria gametocytes are not seen until the seventh day and do not become infective to mosquitoes until the tenth day of the illness. In relapses, on the other hand, gametocytes are usually seen on the first day on which the occurrence is reported. Of great importance is the number of feeds; unless the patient is an exceptionally good infector of anopheles it is necessary to feed the mosquitoes several times in order that sporozoites may be found in the salivary glands. Human blood is the only satisfactory food; certain fruits are unfavourable to oöcyst development. The length of life of the mosquito is another very important point; *maculipennis* can withstand very low temperatures, but its mortality above 22° C. is high. Sporozoites were not killed when the mosquito lived for

three weeks at 4° to 5·5° C. Growth and development of oöcysts is arrested at low temperatures, but begins again when the temperature becomes sufficiently high. These observations support the findings of Swellengrebel in Holland, Wenyon in Macedonia, and Sella in Rome. There is now no doubt that *P. vivax* can be carried through a severe winter in hibernating mosquitoes and the carriage may be in the oöcyst or sporozoite stage, or in both. The infected mosquito carries zygotes at different stages of development; these zygotes rupture at different times and the salivary glands are constantly being replenished with sporozoites. As a result of his observations on the transmision of malaria from mosquitoes to man, James came to the conclusion that relapses were not more frequent in patients infected by a large dose of sporozoites or by infection on several occasions. The qualities of the blood and of the tissues of the individual are much more important than the degree or frequency of infection. There seems to be no doubt that some persons are extremely susceptible to infection and others are relatively quite refractory.

Reviewing the laboratory work and assuming that what happens in artificial laboratory conditions will also happen in nature, James concluded that of the vast numbers of anopheles mosquitoes which exist in malarious places only a few become transmitters of malaria; he explains the fact that most of the inhabitants of the places become infected with the disease as being due to a mosquito retaining its infection for long periods and being able to infect many people. He found that some of his mosquitoes after biting thirty to forty patients still had numerous sporozoites in the salivary glands, because these were replenished with sporozoites from oöcysts ripening at different times. He thinks that the reason why in nature so few mosquitoes become transmitters of malaria is that they do not become infective unless they have an opportunity of feeding several times every day on an infective patient and take no food other than human blood. The role of malaria-transmitters is therefore reserved for only a few individual mosquitoes which pass their life in a favourable environment, and in a manner very different from that of the remainder of the brood. If this view is accepted the secret of successful control of malaria must be in a particular and exact knowledge of the few individual mosquitoes which are likely to get into a special environment. An essential point in the behaviour of these mosquitoes is that instead of flights in the open-air they pass most of their life in the particular house in which they first settled, and sheltered in some particular dark corner they night after night gorge themselves with blood taken from some occupant of the room. James considers that his laboratory work confirms the view of many epidemiologists that in Europe the dwelling is the laboratory where malaria infection has its origin and is cultivated. This was one of the reasons that led the Malaria Commission of the League of Nations to advise that in the European countries which they visited it would be best to deal with endemic malaria by measures which would be limited to affected individuals

and to the interior of the houses in which they lived. In support of this recommendation it is pointed out that Stephens and Christophers working in Africa in 1899 and 1900 gave striking examples of fever houses in which infection persisted for weeks or months.

The persistence of infectivity in mosquitoes through the winter months explains some of the occurrences in Northern Europe of primary attacks of malaria during that season and in early spring.

The clinical observations at Horton of the induced disease showed that n a primary attack of benign tertian malaria there is an initial stage of gradually increasing irregular fever lasting from two to five days. The fever is remittent at first, but towards the end of the stage it is intermittent. In the developed stage which follows the primary stage the fever is quotidian in 80 per cent of the cases; this stage lasts ten days. The terminal stage is characterized by the fever changing from quotidian to tertian type. On examining the blood of a patient bitten only by one mosquito James found that though at first the parasites showed only one stage of development, in later examinations on the second and third day of fever parasites in two or three stages would be found. The irregular fever is probably the clinical expression of these different groups of parasites sporulating at different times. The group which is least numerous gradually disappears, and towards the end of the attack there is only one group of parasites left, and the temperature assumes the tertian type.

In a second attack resulting from a new infection by mosquito bites the fever shows a true tertian periodicity. There is no stage of quotidian fever. The parasite findings resemble those of a primary attack. But after four or five typical tertian paroxysms a second group of parasites may exert their influence, and the fever then changes from tertian to quotidian type.

A relapse usually has the clinical characters of a second attack, but gametocytes can be found earlier than in a fresh infection.

Though there is such a striking difference between the clinical type of the second attack and the first attack, James does not think that the quality which the patient acquires is necessarily that of possessing immune bodies, which if present in sufficient amount destroy the parasites, as suggested by Yorke and Macfie; it may be due to a modification of the normal chemical reaction of the blood or a change in its content of lecithin or other proteins.

Gordon Thomson states that while attempts to demonstrate the presence of protective antibodies have so far been unconvincing, the evidence derived from serological and other studies tends to support the view that such antibodies do exist. He believes that phagocytosis undoubtedly plays an important part as a controlling factor in malaria infections of man. It may be observed in the peripheral blood and also in smears of internal organs and is not confined to the ingestion of free pigment and dead parasites.

In August, 1927, a study of malignant tertian malaria was begun. A tentative trial of malariatherapy with *P. falciparum* was commenced for the treatment of those patients who had not benefited greatly from a course induced by one or other of the benign parasites. Three strains of *P. falciparum* from India, three from Sardinia, three from Rome, and two from West Africa were employed in these investigations.

Two problems were of outstanding interest: (1) Was there more than one species of *P. falciparum*, and (2) if so, did they vary in malignancy?

James and his co-workers could not find any morphological differences in the strains from India, Sardinia, Rome or West Africa. Only one species seemed to be concerned, and it corresponded with the classical description of the Italian malignant tertian parasite, *P. falciparum*. In 1898-99 Koch came to the same conclusion from his studies in Italy, Africa, America, and the Dutch West Indies. The idea that there are several species seems to have arisen from a study of the clinical observations which appeared to show that there was a separate quotidian form as well as the classical Italian tertian form. Marchiafava and others agree that it is very difficult to make a differential microscopical diagnosis between the supposed quotidian and the tertian parasites. James thinks it is not unlikely that another malignant species will be discovered, though at present the existence of the quotidian parasite has not been definitely proved.

The work at Horton enabled a definite opinion to be formed as regards the second problem. The geographical races, while not being morphologically different, could be recognized as distinct by their clinical virulence, immunological reactions and other biological properties.

It was found that the cases infected with the Rome or Sardinia strains were much more severe than those infected with the Indian strains. The dose of quinine required to control a primary attack caused by the Rome or Sardinia strains was eight times greater than that required for a primary attack caused by the Indian strains. The cases in the Rome and Sardinia groups also continued to relapse for a much longer period than the Indian cases. It was also found impossible to infect the English *maculipennis* with the Indian strains of *P. falciparum*, but when similar experiments were made with the European strains from Rome and Sardinia, batches of infected mosquitoes were prepared without difficulty. Wenyon in Macedonia, Roubaud in France, and Misseroli in Italy also succeeded in infecting a European race of *maculipennis* with European strains of *P. falciparum*. James states that he cannot find in the literature any instance in which a European race of *maculipennis* has been infected with an Indian or African strain of *P. falciparum*.

The temperature curve in malignant malaria was carefully studied at Horton and it was recognized that a remarkable feature of the disease was the presence of two, three, or even more groups of the parasite which continue to sporulate at their regular times without being suppressed by one or two predominant groups. This is the most important respect in which

the malignant parasite differs from the benign parasites *P. vivax*, *P. malariæ* and *P. ovale*.

Clinically malignant tertian malaria is an acute disease consisting of a severe primary attack followed by several less severe recrudescences occurring at relatively short intervals. In this respect it differs from benign tertian malaria in which there are in addition to recrudescences, much later manifestations, which James calls relapses, viz., return of fever and parasites within eight to twenty-four weeks, and recurrences in which fever and parasites return later than twenty-four weeks.

As a result of his observations James considers that the following conditions influence the therapeutic effect of quinine on the clinical and parasitological course of malaria: (1) the mode of infection, (2) the degree of susceptibility to malaria of the patient, (3) the degree of tolerance or immunity which the individual had acquired before the commencement of the quinine treatment, (4) the species of parasites concerned, (5) the virulence of the particular geographical race of the parasite, (6) the dose of infection.

The degree of immunity or tolerance is important and has a great influence on the subsequent course of the disease. The first recrudescence is less severe than the primary attack, and each subsequent recrudescence still less severe until the patient becomes so tolerant that, though parasites may be still present in the peripheral blood, there are no clinical signs.

All therapeutic trials of any system of quinine treatment must be on cases which are in exactly the same stage of the disease. It is often stated that malignant malaria is easier to treat in hospitals in England, the reason being that the cases are always recrudescences, never primary attacks.

The necessity of investigating separately the therapeutic effect of quinine on each of the different species of parasite is now everywhere insisted upon. The same strain must also be used.

As regards the dose, James found in general that primary attacks of cases infected by the bites of many mosquitoes were more difficult to cure and of longer duration than are primary attacks of cases infected by the bites of one or two mosquitoes.

In a later paper, James stated that out of 18,000 female mosquitoes only 5,862 were available for infecting patients. The strain of *P. vivax* obtained in May, 1925, was still being used in 1931, and had not changed as regards its morphological characters or virulence. Out of 1,356 patients, 985 developed benign tertian malaria.

Patients who had a "spontaneous recovery" from the primary fever were found to be still infective in the following fever-free interval, as parasites were still present in the blood. But after a spontaneous recovery from a recrudescence patients were not infective to mosquitoes, as there were not sufficient gametocytes present in the blood.

The infectivity of a case is not now judged by the number of gametocytes,

but by (1) the number of exflagellating males with reference to the number of leucocytes, and (2) the time required for complete exflagellation in a saturated atmosphere at 25° C. The number of zygotes in the stomach of the mosquito is increased by the number of feeds; in heavily infected insects the zygotes are distributed almost equally over the whole stomach and in the lightly infected they are present towards the posterior end only.

Of batches of mosquitoes prepared in April and May, less than ten per cent lived until the batch became infective, but of batches prepared between August and November, at least fifty per cent of the mosquitoes were available for infecting patients.

The finding of sporozoites in the salivary glands of a mosquito after biting is not considered to be good evidence that sporozoites were injected by that mosquito when it bit the patient. It is suggested that the sporozoites, in addition to being present in the salivary glands, must be lying free in the common salivary duct of the mosquito at the time of biting. Some of the failures to infect patients in 159 cases were attributed to this cause. Others might have been due to anæmia, the blood not being a good medium for the malaria parasite.

Failures may also be due to previous attacks. When there has been a recurrence followed by "spontaneous recovery," endeavours to bring on a fresh attack by re-infection with the same parasite fail.

Immunity to re-infection by *P. vivax* did not confer protection against infection by *P. falciparum*. An even more remarkable finding was that complete immunity to re-infection by one strain of *P. vivax* did not confer protection against another strain of the same species.

The records at Horton contained cases in which the expected malaria attack did not develop until six months or more after the patient was bitten by infected mosquitoes. The infection remained latent. Swellengrebel, De Buck and Swellengrebel de Graaf have reported a similar series of cases in healthy persons who volunteered for the experiment.

In primary infections, a concurrent mixed infection with two species of the malaria parasite cannot often be demonstrated, because one species quickly becomes predominant and the other disappears until the attack caused by the predominant species is over. Benign tertian seems to predominate over both quartan and malignant tertian.

The spring rise in the seasonal incidence of natural tertian malaria is considered to be due to recurrences in persons who had their primary attack in September, together with primary attacks in persons whose infections in September remained latent throughout the winter.

The possibility of prophylaxis by quinine was tested by James. He gave quinine in various ways in an endeavour to kill the sporozoites injected by mosquitoes and the trophozoites resulting from them during the incubation period. Quinine given before infection and daily during the incubation period did not kill the sporozoites, but it prevented parasites appearing in the peripheral blood for eight and nine days after the commence-

ment of the daily fever paroxysms, which appeared when the prophylactic doses were stopped. In a second experiment, quinine was given daily and continued for a long time after the expiry of the incubation period. A mild clinical attack, lasting only two or three days, appeared at the end of the incubation period; at the end of a fortnight another mild attack appeared, and so long as the person continued to take the prophylactic dose of quinine these mild clinical attacks occurred at irregular intervals. If a person neglected to take the dose he went down with a frank clinical attack in three or four days. In the third type of experiment, a larger dose (fifteen grains) was given once a week; this was not so effective as taking a small daily dose.

When infection takes place by direct blood-inoculation, as in the experiments reported by Yorke and Macfie, quinine given before and after inoculation is quite effective. In these cases, only the forms which live in red corpuscles are injected. In the natural method of infection, the parasite has lived in the walls of the stomach and the salivary glands—in tissue cells—and has not been dependent on blood for its nutriment. Evidently, quinine has not the same action on a tissue parasite as on the blood-cell forms. Relapses and recurrences are rarely seen in the inoculated cases, but occur in fifty per cent of the mosquito-infected cases. What happens to the sporozoites in the long delayed cases? It has been supposed that they enter the connective tissue cells or the cells lining the capillary blood-vessels, and remain there until the cells break down eight or ten months later. Another explanation offered is that the sporozoites actually penetrate the blood-corpuscles as Schaudinn described, while the merozoites are merely attached to the corpuscles.

The practical point as regards quinine prophylaxis is that while the parasites are not killed, the clinical manifestations are suppressed, so that bodies of men such as troops in the field, crews of ships, or labourers on particular work, can carry on their duties for a limited period, which may be of prime importance from a Service point of view. James believes that when quinine is taken there is not the same immunity as appears after recurring attacks of malaria, and in exceptional circumstances, such as very severe work or prolonged exposure to the sun, the men may suffer from a severe clinical attack of fever.

Evidently no general rule can be given and whether quinine should be given prophylactically will depend on the particular circumstances, especially the frequency with which men are subjected to infection and re-infection.

The problem of the synthesis of anti-malaria preparations has been investigated for some years in the Bayer-Meister-Lucius Research Laboratories at Elberfeld by Dr. Schulemann and his colleagues.

The assessment of the value of these preparations was made possible by the method of using canaries worked out by Rochl in 1926. The birds

were infected by direct blood inoculation. In October, 1931, Colonel James visited Elberfeld and suggested that it was necessary to transmit the malaria from one bird to another by means of the mosquito and not by direct injection of infected blood. Several strains of the proteosoma type of avian malaria parasite must also be used; and tests must be made with the halteridium type of parasite of which the asexual cycle is passed in endothelial cases. Dr. Kikuth immediately instituted tests on these lines. On the basis of toxicological and chemo-therapeutic properties plasmoquine was selected for tests in 1925. In naturally acquired human malaria the action of plasmoquine was demonstrated by Rochl, who was the first to discover that it destroyed the gametocytes of *P. falciparum* within a few days. The work of Sinton, Knowles, Wallace and Manifold has demonstrated that with plasmoquine combined with quinine it is possible to reduce the relapse rate of tertian malaria from fifty per cent. to between two and five per cent. Quinine acts effectively on the schizonts of *P. falciparum* and allays the acute clinical symptoms, plasmoquine has practically no effect on the subtertian schizonts but it definitely destroys gametocytes in a few days. The idea that plasmoquine can inhibit the formation of gametocytes has not been confirmed. The work of Barber, Komp and Newman, Withmore, Roberts and Jantzen in Central America, has, however, definitely proved that even minimal doses of plasmoquine, which are too small to cause the gametocytes to disappear, nevertheless render them incapable of infecting anopheles.

James made a prophylactic experiment with plasmoquine at St. Mary's Hospital and concluded that it is a true prophylactic, but that the daily dose requisite for ensuring complete protection in all circumstances would be too near the toxic dose to be safely taken for more than a few days. What is required is a drug with the same action as plasmoquine but of less toxicity to the human host.

James and his co-workers treated seventeen cases of malignant tertian fever with 0·3 g. of atebrin for five days; in only one case was there a failure to effect a permanent cure, and even in this case further treatment with the same dose for seven days was followed by quick recovery and no further recrudescence. All the cases were under observation for several months. In each case arrangements were made to eliminate the errors in chemo-therapeutic trials previously noted. The strains of *P. falciparum* employed were either from Rome or from Sardinia. Quinine had been proved to be so ineffective against these particular strains that permanent cure by its means could not be brought about.

There is urgent need for further research on the life history of sporozoites in the human and insect hosts, for James's experiments show that plasmoquine is more effective for preventing malignant tertian than it is for preventing benign tertian malaria. He deduces from his work that malaria must be studied in terms of the different species of parasite concerned; and in terms of the various phases of each parasite.

There must be a sporozoite therapy, a schizont therapy, and a gametocyte therapy. Moreover, these subjects must be studied in terms of geographical strains of the different species.

Antimalaria chemotherapy has thus become an exceedingly complex research problem. But we must not come to decisions on laboratory trials alone; it is important to determine whether an antimalaria drug has the same action on a particular strain occurring in an indigenous native in the tropics as it has on the same strain occurring in a European in England. Professor Schulemann has sent Colonel James a record of clinical trials in Africa which indicate that among natives in that country plasmoquine in a daily dose of only 0·02 g. may be an effective prophylactic.

These results and those obtained by Barber and others in Central America may possibly explain the reduction in malaria observed by Colonel Hanafin in Burma.