LEAGUE OF NATIONS

CH/Malaria/201.


HEALTH ORGANISATION.

Malaria Commission

The Medical Director has the honour to communicate herewith a Note on

THE STANDARDIZATION OF MIXED PREPARATIONS OF THE CINCHONA ALKALOIDS IN RELATION TO INDIAN CONDITIONS

by Lt. Colonel J.A. SINTON, M.D., D.Sc. I.M.S.

(Director, Malaria Survey of India, KASauli, Punjab).

----------

This document has been prepared for the meeting of the Study Committee of the Malaria Commission. It is purely the personal opinion of Col. Sinton on the subject and is not to be considered as an official statement from the Government of India.

----------

The Malaria Commission of the League of Nations in their meetings in 1929-1931 studied carefully the information available as to the relative values of the different crystallizable alkaloids of Cinchona bark. As a result of their deliberations it was decided to recommend that the present mixed preparations of these alkaloids (cinchona febrifuge, total alkaloids, quinetum etc.), should be replaced as far as possible by a standardised preparation to be known as "Totaquina". The composition suggested for this new product was as follows:

"This preparation should contain at least 70 per cent of crystalline alkaloids, of which not less than 15 per cent must be quinine. Amorphous alkaloids should not, however, exceed 23 per cent, mineral matter 5 per cent and water 5 per cent".

In this age of scientific medicine, the standardization of the contents of any drug used in human therapy is undoubtedly
a proper procedure. It seemed reasonable, therefore, that products of such varied composition as the preparations of the mixed cinchona alkaloids should be brought into line with other therapeutic preparations, if they were to take their places in the Pharmacopoeia of the world, either as a substitute for quinine or as additional weapons in the fight against malaria.

Large amounts of cinchona febrifuge are manufactured by the Government Cinchona Factories in India and the drug is widely used in that country. It is therefore necessary to consider carefully, in relation to malarial conditions in India, –

(a) The reasons for the displacement of cinchona febrifuge and quinetum by a standardised preparation of the nature of totaquina.

(b) The uses to which it is proposed to put the new drug and

(c) The possibility of preparing a cheap totaquina.

(d) The material benefits which such a change might confer on the malarious population of India.

If, from a consideration of these various factors, it be definitely decided that the change from cinchona febrifuge to totaquina will confer a distinct practical benefit upon the malarious population of India, there is no doubt that such a change should be made.

REASONS FOR THE ADOPTION OF TOTAQUINA.

It is an axiom well known to all malariologists, that the treatment of the malarious sick should be the first step in any anti-malarial campaign. There are not, however, enough anti-malarial drugs produced annually in the world to treat more than a tithe of the people who suffer from the disease. On the other hand, the world stocks of the main anti-malarial drug, quinine, have been accumulating steadily, partly because
of the difficulties in placing it in the hands of the 
sufferers, and partly because Governments cannot afford to 
purchase the amounts needed to treat all their sick. 
Malarious countries are usually poor and the cost of even a 
limited mass-treatment campaign is often prohibitive, if 
quinine be the drug of choice for such measures. 

The Malaria Commission of the League of Nations considered carefully the various means whereby the prohibitive cost of anti-malarial drugs could be lessened, and the supply of anti-malarial drugs increased, with resultant benefit to the poorer sufferers in malarious countries.

The evidence at present available goes to show that all the crystallizable alkaloids of cinchona bark have a definite therapeutic effect on the manifestations of malarial disease. Although there may be some difference of opinion as to which of these alkaloids has the most powerful therapeutic action, there can be no doubt that the crude mixture of these alkaloids obtained from cinchona bark are very useful drugs in malarial therapy.

The Malaria Commission having considered the available evidence, decided that this was sufficient to show that some of the cheaper preparations containing all the crystallizable cinchona alkaloids, would give satisfactory clinical results. They therefore recommended the use of these mixtures for mass treatment, more especially in countries which could not afford to buy other anti-malarial drugs in the very large amounts necessary to treat all their sick.
Having arrived at this conclusion, analyses of the different alkaloidal mixtures at present in use, showed that these had a very varied composition. It was therefore decided that a mixed alkaloidal preparation with a standard composition *(vide supra)*, should be recommended for adoption by the National Pharmacopoeia of different countries.

There is no doubt that the adoption of such a standardised drug would be very valuable, by

(a) Preventing the sale of markedly adulterated preparations of the cinchona alkaloids or those with little therapeutic value.

(b) Maintaining a standard content of the effective alkaloids, and a diminution of those which appear to be of little therapeutic value, or even harmful in character.

(c) Ensuring that therapeutic results obtained by such mixed alkaloids would be relatively constant and would also be comparable from a scientific point of view and

(d) Providing a safe, reliable and cheaper drug in the treatment of malaria, for wider use in those countries which cannot afford quinine in sufficient quantities to treat all their sick.

USES PROPOSED FOR TOTAQUINA.

It was not proposed that this new standardised preparation should displace quinine or other drugs of proved value in the treatment of malaria. The intention is that a good and cheaper treatment will be made available to supplement the supply of these drugs. If this can be done, it should
be possible to make great extensions in treatment campaigns in many countries, whose activities in this respect are now hampered by lack of funds. In the tropics it is probable that at the present time, not more than one-tenth of sufferers from malaria ever receive any specific treatment.

In many tropical countries wide-spread anti-malarial measures are financially impossible and the chances of re-infection are high. The best results that can at present be hoped for from treatment under such circumstances, are that the clinical manifestations of attacks of the disease will be alleviated and the lives of the patients saved, in the majority of cases. It is, therefore, necessary to discuss in how far totaquina or other mixed alkaloidal preparations will meet the requirements of such a situation.

The properties which it is considered that any drug should possess for the ideal treatment of malaria have been detailed elsewhere (Sim\'ton, 1930). These factors must therefore be discussed in so far as they affect mass treatment with totaquina.

(a) So far as can be judged from the evidence available, such a mixture may possibly be slightly slower in its action, but for all practical purposes should be very little inferior to quinine, in controlling the clinical manifestations of the disease*. Such a cure of clinical symptoms is what the patient demands primarily.

(b) Many years of experience has shown that the cinchona alkaloids can be safely placed in the hands of the lay public for medical purposes.

* This applies solely to the oral administration of the drug, which is the only method applicable under the conditions of mass-treatment for which totaquina would be most widely used.
(c) It has been the experience of most workers in the tropics that the uneducated, and often the educated, patient will stop treatment with any of the cinchona alkaloids as soon as clinical symptoms have disappeared, unless compelled to continue. Apart from the question of reinfection, it cannot be hoped with treatments of such short duration, to produce a permanent cure in any large percentage of patients, whether quinine, cinchona febrifuge or totaquina are used. For the cure of clinical symptoms, preparations of the mixed alkaloids would seem to be almost as effective from a practical point of view as quinine.

(d) While the cinchona alkaloids will destroy the gametocytes of \textit{P. vivax} and \textit{P. malariae}, they have little affect on those of \textit{P. falciparum}, usually the commonest parasite in the tropics. No one alkaloid has been proved markedly more effective in this respect than any other. It is here that drugs like plasmoquine have proved to be superior to the cinchona alkaloids.

(e) There is some evidence that quinine may have a greater effect in the production of a permanent cure in malignant tertian malaria than in the other infections. There is, however, no conclusive evidence that, when given over the short periods mentioned above, the results will be markedly more successful than with the mixed alkaloidal preparations. The standard of 15\% recommended for totaquina, however, ensures that a considerable amount of quinine will be present in this drug.

(f) Additional factors in relation to mass treatment:

(i) It is cheaper in price than quinine. (ii) With some samples of the mixed alkaloids the taste is said to be slightly more disagreeable than that of quinine, and symptoms, such as nausea and vomiting, more common. These unpleasant effects are probably due to the content of amorphous alkaloid, about the therapeutic value of which considerable doubt exists. In the standardised preparation it has therefore been laid down that such amorphous alkaloids should not exceed 20 per cent. (iii) It requires little expert supervision. (iv) As noted previously, it cannot
tC hoped that totaquina will produce a permanent cure in a few days in the majority of patients, but in this respect it is probably little inferior to quinine.

Several of the newer drugs, such as plasmoquine and atebrin, fulfil some of the conditions of an ideal treatment where the cinchona alkaloids fail, but even these drugs do not fulfill all the conditions. For the present they can only be looked upon as adjuvants, but very important ones, to treatment with the cinchona alkaloids. Unfortunately the cost of these drugs, and in some cases the very narrow limit between their therapeutic and toxic doses, makes them unsuitable for mass treatment, except under very special conditions of medical supervision, which are seldom available in most areas in the tropics.

From the data given above, it would appear that a mixed alkaloidal preparation of the nature of totaquina, would probably be little, if any, less valuable than quinine for mass treatment, as carried out in the tropics.

THE PREPARATION OF A CHEAP TOTAUQUINA.

Extensions of Cinchona Cultivation.

Mixtures of cinchona alkaloids have been in use for many years in the form of substances such as cinchona febrifuge, residual alkaloids, quinetum, etc. The price of such drugs is less than that of quinine, because the method of manufacture is less costly and because, in some instances, quantities of the more fashionable and expensive alkaloid, quinine, have been extracted from the crude mixture. The cost is also lower, because the bark employed for their manufacture is sometimes one which has a low quinine content and is, therefore, less profitable to use for the extraction of the latter drug.

The original Indian cinchona febrifuge, quinetum and quinin were mixtures of the total alkaloids from the bark of species of cinchona like C. succirubra. The present Indian cinchona febrifuge is a mixture of the residual alkaloids, left after most of the quinine has been extracted from the quinine-rich bark
of *C. ledgeriana*, and to which residue sufficient quinine has been added to make its composition very similar to that of the original febrifuge. This change was necessitated by the scarcity of *C. succirubra* bark following upon the abandonment of cultivation of this tree by private planters in India. This was due to the marked fall in price of quinine, resulting from the great success which followed the cultivation of *C. ledgeriana* in Java.

The high price of quinine depends largely upon the fact that the tree which gives the highest yield, *C. ledgeriana*, will only flourish under very special conditions of climate and soil, which are only found in very limited areas. Although the hardier species, like *C. succirubra* and *C. robusta*, will flourish over much wider areas, their yield of quinine is too small to make their cultivation a commercial proposition, if, as at present, quinine remains the chief drug to be used for malarial treatment. With the wider adoption of the other alkaloids in the treatment of malaria, the cultivation of such trees might again become commercially profitable for the production of the cheaper drug totaquina. The hardier nature of these trees might even make it possible for most of the malarious countries in the tropics to produce their own supplies of this drug at a low cost. This eventuality was considered when the Malaria Commission fixed the standard for totaquina at approximately the total alkaloid content of the bark of such trees.

If bark of this nature were now available in sufficient amounts, the cost of totaquina should remain low. Unfortunately, it seems that the supply of such cheap bark is at present small, as judged by the potential needs of such a widespread campaign as is suggested. When this is exhausted the demand for totaquina will have to be met chiefly by the use of bye-products from the *ledgeriana* bark used to produce quinine, until such time as new bark becomes available. Under the latter circumstances, it seems doubtful whether a totaquina of the high standard suggested can continue to be produced at the low price anticipated.
To prevent such a rise in price of totaquina, it would be necessary to extend very widely the cultivation of the hardier Cinchona trees, to meet the demand for cheap bark. The object of such plantations should not be to supplant those cultivated for the production of quinine, but to supplement the supply of anti-malarial drugs for the needs of the poorer sufferers of malarious countries.

It must be remembered, however, that such new plantations will not yield bark for a considerable number of years, so in the meantime it seems possible, as suggested above, that the price of mixed alkaloidal products of the standard of totaquina will rise.

While from a medical and agricultural point of view the extension of cultivation of the hardier species of Cinchona, seems a most laudable object, the policy apparently involves much wider issues than these. Such a change in policy might almost be compared to a departure from the gold (quinine) standard.

Various questions will need to be discussed fully and carefully before such a step is taken.

(a) Will some new synthetic drug be discovered in the near future which will be cheaper and equally efficacious for the use envisaged for totaquina?

(b) Will it be possible to meet the demand for totaquina in the near future at the present low price, taking into consideration the supply of cheap bark available?

(c) What effect will the increased demand for the other alkaloids have on their price as compared with quinine?*

(d) Will a fall in the price of quinine make such new plantations commercially unsound or unnecessary in the future?

* The Madras Commission of 1866–1867 reported that certain of the other alkaloids of cinchona bark were as good as quinine for the treatment of malaria. The result was that the price of these alkaloids immediately rose to be equal or even greater than that of quinine.
Possible Effects of Standardization on the Present Price of Indian Cinchona Febrifuge.

There is no doubt that the cinchona febrifuge used in India may vary very considerably in its composition from time to time. That such a variation should occur, is not to be wondered at, when one takes into account the fact that these mixed alkaloids are mainly manufactured as a bye-product in the extraction of quinine from cinchona bark of very diverse alkaloidal content. Under such conditions some standardization of the mixed alkaloidal product would seem essential.

When however one considers that the low cost of cinchona febrifuge in the past, has depended to some extent on the absence of any complicated process of standardization, one is led to enquire whether such a standardised product could be manufactured without a considerable increase in cost. Such an increase, if considerable, might neutralise largely the value of this drug as compared with quinine. i.e. the difference in price.

From the information available at present, it would seem that a change in the present system of manufacture of cinchona febrifuge, so that it should be replaced by totaquina, would result in an increase in the cost of production. The preparation of such a standardised mixture would require more complicated process, more elaborate analyses* and a greater addition of some of the more expensive crystallizable alkaloids, if, as at present, it is only manufactured as a bye-product in the extraction of quinine. It is impossible to say at the moment how much this extra cost will be, or how far such an increase in price would annul the advantages of the present low cost of such mixed alkaloids.

* There also seems to be no very close agreement between the analyses of this substance made by various chemists, even in regard to the points on which the Malaria Commission has laid stress.
All these points would require very careful consideration by experts, medical, financial and agricultural, before any extensive changes were made in the present methods of manufacture of mixed alkaloidal products in India.

THE BENEFITS ARISING FROM THE REPLACEMENT OF CINCHONA FEBRIFUGE BY TOTAQUINA UNDER INDIAN CONDITIONS.

At present most of the cinchona febrifuge and similar alkaloidal mixtures used in India are manufactured by Government Departments. Under such circumstances, there seems little likelihood that these products will fall much below their present total crystallizable alkaloidal content. If, however, the manufacture of such products were to be taken up by commercial firms in India on a large scale, or large amounts of these drugs were imported from very varied sources, the need for a standard composition would at once be urgent.

Such a standardization of the Indian cinchona febrifuge would result in an increase in the amount of crystallizable alkaloids and a diminution in the percentage of the amorphous ones. The former change would probably be beneficial by making some increases in the rapidity of clinical cure, which might also be obtained by a slight increase in dosage. The decrease in the percentage of amorphous alkaloids would probably be advantageous by causing a diminution in the nauseating effects produced by these alkaloids. These effects would also tend to be augmented by any increase in the dosage as suggested above, so some diminution in these alkaloids seems essential.

For the reasons mentioned, the presence of a high content of crystallizable alkaloids combined with a low one of amorphous alkaloids, would seem to be the objective to be aimed at in any standardized product for mass distribution. Even
if at present a product of the standard of totaquina could not be produced in India, at a price which would maintain its practical value in comparison with quinine, it might be possible to remedy some of the defects of cinchona febrifuge to a considerable extent, without materially affecting its cost.

From a consideration of the probable therapeutic effects of quinine and cinchona febrifuge when used for mass treatments, it seems doubtful whether totaquina would have any very extraordinary advantage over such an improved cinchona febrifuge manufactured in the Government Factories in India.

If the price of such a preparation should turn out to be much greater than that of the present cinchona febrifuge, it seems possible that more beneficial results to the population might be obtained at the same cost by a wider use of the latter product than a more limited one of the former. Even a rise of half an anna per patient per annum in the cost of treatment of each individual among all the estimated 200 millions, who suffer from malaria each year in India, would mean a total sum of nearly half a million pounds sterling. It is not a question of what is the best drug procurable, but of what drug will give the greatest benefit to the greatest number for the money available.

The necessity for the standardization of a mixed alkaloidal preparation seems to me a question which is at the moment of much more urgent import under European conditions than in a country like India, which manufactures its own product.

In Europe (i) the facilities for a widespread distribution of treatment are much greater than in India or other tropical countries; (ii) the chances of reinfection after cure are usually less, and many more people will continue their medicine after clinical symptoms have abated, so it is necessary
to have as effective a drug as possible to increase the chances of permanent cure; (iii) the actual number of malarial cases is small, compared with the millions in India, so slight differences in cost between cinchona febrifuge and totaquina would not be so important; and (iv) countries buying drugs of such variable composition, from a large number of different sources, must necessarily insist upon a standard composition.

In India, on the other hand, the most urgent problems which confront the Government at the moment are not the standardization of cinchona febrifuge, but (i) how can the present available supply of antimalarial drugs be used to produce the greatest benefit to the greatest number of sick; (ii) how can these drugs be most easily and cheaply placed within the reach (both financial and physical) of the afflicted population; (iii) how can such a distribution of these drugs be continued in future and (iv) how can the cost of these drugs be reduced?

Under such circumstances it would seem that, however desirable the production of a standardized preparation of the mixed cinchona alkaloids may be, it is not of such urgent importance to India at the moment, as it is to many other countries.

While admitting the importance of a standardized preparation, this question should not be allowed to assume such proportions that it obscures the other more immediately urgent problems relating to wider facilities for treatment among the malarious masses in India.

As the question of increase in the area of Cinchona cultivation must necessarily be settled many years before the demand for the bark comes, this problem would need to be settled in the near future. This does not, however, seem possible until many of the points raised in this note are settled by a Conference thoroughly familiar with the very varied aspects of the problem.
CONCLUSIONS.

1. A standardization of the alkaloidal contents of mixed alkaloidal preparations of cinchona bark is to be encouraged.

2. Such preparations seem eminently suitable for mass treatment among malarious populations who cannot afford more expensive drugs and who cannot easily obtain medical advice.

3. Before the manufacture of the present cheap alkaloidal mixtures are replaced by that of such standardized preparations, it is necessary to consider carefully (i) what effect this will have on the present cost of such products; (ii) what effect it will have on the present policy of cinchona cultivation and (iii) how many years it will take to grow sufficient bark from such hardy trees as C. succirubra and C. robusta, to meet the demand for bark to manufacture a cheap and adequate supply of a total alkaloid preparation of the character mentioned.

4. Serious attempts should be made to improve the Indian cinchona febrifuge by an increase in total crystallizable alkaloids and a diminution in the amorphous ones, if this can be done without any very great increase in cost.

5. The more urgent problems, which at the moment confront India in connection with malarial treatment, are connected with the distribution of treatment to the malarious masses of the country, rather than the standardization of cinchona febrifuge.

***************

S.F.

1.3.33.
LEAGUE OF NATIONS

CH/Malaria/202


HEALTH ORGANISATION

Malaria Commission

The Medical Director has the honour to communicate herewith a Report on:

RICE CULTIVATION IN SPAIN WITH SPECIAL REFERENCE TO

THE CONDITIONS IN THE DELTA OF THE RIVER EBRO

by Lt.-Colonel J.A. SINTON, M.D., D.Sc., I.M.S.
(Director, Malaria Survey of India, KASUALI, Punjab.)

This document has been prepared for the meeting of the Study Committee of the Malaria Commission.

INTRODUCTION.

Through the kindness of the League of Nations I was given an opportunity of visiting some of the rice-growing areas in Spain during the summer of 1931.

The duration of my tour was necessarily short, and it was impossible in the time at my disposal to become au fait with the many aspects of the malaria problems which occur in connection with rice cultivation in that country. Thanks to the valuable assistance given me by Dr. Gil Collado, Dr. Torredem, Dr. Peréperez and Dr. Serra, very much valuable information on the local malarial conditions was placed at my disposal. Dr. Pampana of the League of Nations accompanied me on my tour, and I am very grateful to him for all the help he gave me.

It is with some diffidence that I presume to give my impressions of the problems seen, but I do so in the hope that some of my suggestions may be helpful to other workers.
The main areas of rice cultivation visited were those at San Fulgencio (Alicante), the Gandia-Valencia area, the Prat el Llobregat near Barcelona and the Ebro Delta.

**RICE CULTIVATION IN SPAIN.**

The problems of malaria in connection with rice cultivation in Spain, show a considerable difference from most of the problems with which I am familiar in connection with this form of agriculture in India. Aggregation of labour seems to play a very important part in epidemics of malaria in Spain, and only one species of Anopheline*, *A. maculipennis*, is said to be implicated in connection with rice cultivation.

This form of agriculture in Spain is almost entirely confined to the alluvial tracts of deltaic areas, and the malarial problems presented in the different areas visited were very interesting. In some areas of rice cultivation malaria is an important problem, as in San Fulgencio, the "Prat" el Llobregat, and the Ebro Delta. In other areas, however, the incidence of this disease is said to be negligible, as in the Gandia-Valencia region.

During my tour in Spain the localities mentioned above were visited. Unfortunately on account of the dryness of the year, the areas at San Fulgencio and around Valencia were not being cultivated, so it was impossible to make any extensive personal observations in these places. It was possible, however, to make a closer study of the conditions in the Ebro Delta and in the Prat el Llobregat.

Most of the factors influencing the spread of malaria in any region consist of (I) a susceptible human population, (II) a susceptible mosquito population with inclination and

---

*Foot Note. This point has been discussed later in this note.*
opportunities to bite man, (III) the presence of human gametocyte carriers, and (IV) climatic or other conditions favourable for the development of the malarial parasite in the insect host. Taking these factors into consideration, it is interesting to study their apparent relationship to the malarial conditions in rice-growing areas.

It seems possible to divide the history of rice cultivation in the regions visited in Spain into three very definite phases, each of which presents a different aspect:

**Phase I.** The period during which uncultivated and swampy localities are being converted into areas suitable for rice cultivation, i.e., levelling, filling, drainage, installation of irrigation, etc.

**Phase II.** The period during which cultivation is commenced and the people are living under more or less primitive conditions.

**Phase III.** The period when rice cultivation is well-established and the people are reaping in full the reward of their labours.

In addition to the three phases mentioned above, there is possibly another stage - **Phase IV**, during which there predominates a species of Anopheline which has little or no inclination to bite man.

Most of my observations were made in areas where Phase II appeared to be merging into Phase III. The deductions concerning the other phases were chiefly made from the information placed at my disposal by various workers. I was unable to study an area similar to Massarosa in Italy, where Phase IV appears to be acting as the cause of a low malarial incidence. It is possible that this stage may be present in the Gandia-Valencia area.
RICE CULTIVATION (PHASE I)

If rice-cultivation in any new area in Spain be started on a large scale, it appears to be invariably associated with a severe outbreak of malaria. This outbreak affects both the immigrant labour population and the local inhabitants. From the data available the following factors seem to have played, in the past, important roles in the causation of these epidemics:

(A) Aggregation of immigrant labour and the conditions associated with it.

(B) The opening-up of swamps and waste land, the introduction of more water, and the resultant disturbance of the established balance of the original indigenous flora and fauna of the area.

(C) The effects of the changes in population, both human and animal, upon the distribution of Anophelines.

(A) Aggregation of Labour.

This is a very important factor in the production of local epidemics of malaria in tropical countries. A very similar condition appears to occur in Spain, in connection with planting and harvesting operations and the initiation of large agricultural and industrial schemes. Many of the most malarious places visited seemed to be connected with this condition. In some other towns and villages, a greater or lesser proportion of the malarial incidence was attributed to the return of infected labour from such operations. This has caused not only a fictitious increase in figures of locally acquired disease, but also a true increase in these, due to the rise in local infections as a result of the introduction of many gametocyte carriers.

Under conditions of aggregation of labour, the outbreak of malaria may be started in two different ways:

(i) A group of workmen coming from an area where malaria is
slight or absent (i.e. non-immunes), are introduced into a locality where the disease is rife, or

(ii) A group of workmen, from many different places where malaria is prevalent, are introduced into a locality where the incidence of the disease is low, but where the conditions engendered by the work in progress are highly favourable for the spread of the disease (i.e. a great increase in the numbers of carrier Anophelines and of human gametocyte carriers).

In the former instance the brunt of the epidemic will probably fall primarily on the immigrant population, and later affect the local inhabitants. In the latter instance the outbreak will affect both populations eventually, but it is probable that the local or non-immune populations will suffer most in the initial stages.

Sometimes this immigrant population is drawn largely from areas where economic stress is present, because the contractors can obtain cheaper labour in this way. Such cheapness of labour, however, often means that the workmen have to live under very poor conditions of diet, sanitation and housing, because they are unable to afford anything better on the wages received. Apart from the question of wages, the arrangements for the accommodation and feeding of labour are often, of necessity, primitive at the commencement of any large undertaking in a remote locality. These adverse conditions usually mean a lowered resistance of the worker, and poor facilities for the treatment of any infection he may acquire.

Shortage of accommodation, more especially in the initial stages, leads to overcrowding. This may exercise its deleterious effects in a variety of ways:

(i) It facilitates the spread of infections, because one infected mosquito in a room or barrack will be liable to infect more than one person.

(ii) Conversely a larger number of mosquitoes are liable to
acquire the infection from one human gametocyte carrier than if there be fewer persons present.

(iii) The chances of a mosquito acquiring an infection rise with the number of feeds it gets on an infected person. These chances rise with overcrowding.

(iv) The severity of an infection in man appears to increase with the number of bites he receives from an infected mosquito. Multiple bites are much more likely to occur under the conditions mentioned.

(v) The presence of multiple human gametocyte carriers from different areas and of multiple infected insects, makes the chance of infection with a number of strains or species of parasite more probable.

(vi) The close association between the insect and human carriers under such conditions, are ideal for rapid and repeated transmission of the parasite. Such transmission may possibly cause an increase in the virulence of the parasite, although the evidence on this point is not conclusive (Sinton, 1931).

All these factors play important parts in the causation of malarial outbreaks under conditions of labour aggregation. The introduction of a great variety of "foreign" strains of malarial parasite has probably a very important bearing on the severity of the attacks of the disease, and this has apparently a very close relationship with the number of gametocytes produced by any attack (Sinton, 1926).

---

*Foot Note. The probable importance of the strain of parasite in relation to the severity of the malarial attack, and to the rate of clinical or permanent cure of the disease, has been discussed elsewhere (Sinton, 1931). The great influence of this factor in connection with severity of the attack in super-infections by different strains of parasite, has been confirmed by recent researches on monkey malaria in these laboratories.*
When the constructive operations are started and are in progress, the habitations are usually collected into large groups. The location of these groups is determined by the position of available and suitable ground, and its relation to the work in progress. These areas are often chosen near the site occupied by the local inhabitants. This facilitates the passage of infection between immune and non-immune populations. Many of the factors associated with overcrowding also come into action.

If suitable malaria-carrying insects be present, the operations needed in preparing an area for rice cultivation are usually those suitable for a great local increase of such insects (vide infra).

The main factors responsible for those outbreaks of malaria which are associated with aggregation of labour in malarious regions, may be summarised as follows:-

(i) A large non-immune or partially immune population introduced into a highly malarious area, or vice versa.

(ii) A great local increase in the numbers of either insect or human carriers of malaria, or both.

(iii) Poor economic conditions causing a lowered resistance of the population to the disease.

(iv) An increased severity of the clinical symptoms of malaria, with a consequent increase in the numbers of gametocyte carriers and of the numbers of gametocytes in the blood of each infected person. This may be due to -

(i) Lowered resistance of the population.

(ii) Primary attacks of malaria in a non-immune population.

(iii) Introduction of many different strains of parasite, or enhanced virulence of the local strains.

(iv) Greater chances of multiple infections with different strains and species of parasite, as well as an increased dosage of sporozoites due to multiple bites.
(5) Overcrowded accommodation in houses and close grouping of dwellings, facilitating the passage of infection from man to man.

(6) Absence of facilities for good and prompt treatment, and for the nursing of sick persons.

(B) Opening-up of swamps, etc., and Introduction of Irrigation.

Apart from the great increase in the number of breeding-places for mosquitoes caused by such operations, several other factors may come into play.

These factors are chiefly connected with the disturbance of the natural balance, which has been established from time immemorial between the various types of flora and fauna in the area.

During the primary stages of preparation for rice cultivation, the drainage, filling, levelling, clearing, etc., cause marked changes in the local vegetation, not only the terrestrial but also the aquatic kinds. The changes produced, such as exposure of water to sunlight, etc., may result in an enhancement of the breeding conditions of various mosquitoes, and thus an increase in their numbers. The change from the permanent aquatic conditions of swamps, etc., to the intermittent ones of rice cultivation, must necessarily have an influence on the type and abundance of the various aquatic flora and fauna.

The filling-in of swamy areas may result in the destruction of large numbers of the natural enemies of mosquitoes, while the changed conditions of newly-formed collections of water may not be suitable for the survival and multiplication of these enemies.

* Foot Note. The workers make every effort to supplement their scanty diet. Netting and other methods of fishing are largely used, without any regard to the size of the fish captured. This also helps to cause a decrease in the numbers of larvicidal fish.
This will affect the natural balance established between the number of mosquitoes and their enemies, with a resultant benefit to the former.

The destruction of terrestrial vegetation removes many of the places in which mosquitoes naturally shelter. Suitable shelter, more especially in close relationship to a good food supply, is a very important factor in the life history of A. maculipennis. The result is that these mosquitoes tend to crowd into the human habitations, and the latter on account of their primitive character, are usually eminently suited to shelter the insects.

(C). Changes in Population in Relation to Anopheline Distribution.

In the early stages of such work few or no domestic animals are present in proportion to the human population. The flocks and herds, which under ordinary conditions roamed over the area, are taken further afield as their grazing grounds are destroyed or limited by the work in progress. This means that there are few or no domestic animals to divert the attentions of the mosquitoes, with a resultant intensity of their attacks on man.

Preventive Measures in Phase I of Rice Cultivation.

During this first phase of rice cultivation, the operations for which the workmen are collected are of a temporary nature and the groups of workmen in many instances widely scattered. Under such conditions it does not seem that the prevention of malaria by anti-larval measures alone is a feasible financial proposition. Some system of controlled medication, both curative and prophylactic of symptoms, appears in our present state of knowledge to be the most suitable as the basis of prevention under these conditions. If such operations be financed by a wealthy company, the erection of mosquito proofed houses might be considered. These might be built on such a plan, and in such localities, as would make them suitable for later occupation by the new settlers. These buildings would,
however, need to be made prior to any large influx of people, so that many conditions associated with and following upon aggregation of labour, would be less likely to occur.*

RICE CULTIVATION (PHASE II).

When the major work of preparation of an area for rice cultivation is finished, another phase is entered upon.

In this second phase the bulk of the imported labour departs and labour immigration is only present at special seasons to help with agricultural operations such as the planting and harvesting of crops. This seasonal re-introduction of a floating population is necessary, because the number of local inhabitants is not large enough, at this early period of colonisation, to supply the demand for workers.

The original immigrants will have left their legacy of disease behind them and the new comers will tend to prevent this from dying down rapidly.

The social and economic conditions of the local inhabitants at this period will also be conducive to a continuation of a high incidence of malaria. The outlay incurred by them in acquiring their holdings, and the various 'initial expenses in connection with rice cultivation, will leave them in a low financial state in many instances. Such conditions will continue until the pecuniary benefits of several harvests have been felt.

These people will probably occupy at first the old habitations used by the original workmen, until they can build more suitable shelter for themselves. This will make them liable to infection and reinfection from the local mosquitoes. When they build their own houses, these will at first, be small and conditions of overcrowding common.

* At Macarese in Italy, "bonification" along these lines is proceeding.
The adverse conditions of poor food and overwork will continue. Money to buy cattle and house them properly will be scarce, so zoophilism will have little effect. The people will be able to afford little medicine or medical advice, even if this was readily available in a newly-settled area. For the latter reasons efficient treatment will be rare, malarial morbidity high, and gametocyte carriers common.

During this phase, the adverse conditions will resemble those seen in the first phase but to a less degree, while the more favourable circumstances of the third phase will not have commenced to operate to any very appreciable extent.

Preventive Measures in Phase II of Rice Cultivation.

The main scheme of malarial control in this phase would seem to be very similar to that in the first phase, i.e. proper treatment of malarial cases. This should be supplemented, where possible, by a financial subsidy to enable the settlers rapidly to obtain proper housing, sufficient food, a supply of cattle, etc., etc., i.e. a "bonification" such as is being aimed at in the work at Macarese near Rome.

RICE CULTIVATION (PHASE III).

In old and well-established areas of rice cultivation malaria is said to be a negligible problem. This may be called the third phase.

In the years which have elapsed since rice cultivation was started, a poverty-stricken population has become a prosperous one. With this condition of "bonification", most of the adverse influences, which acted as causal factors of high malarial incidence, have disappeared. The people are well-housed in habitations less attractive for the shelter of mosquitoes. Overcrowding has diminished. Screening has been attempted in some dwellings.*

* Foot note. With the large number of suitable and easily accessible domestic animals, even a moderate degree of screening would seem to be sufficient to deter the Anophelines from entering these human habitations in any large numbers.
Over-work and the scarcity of good food have largely disappeared. Domestic animals are plentiful and are housed under conditions which attract mosquitoes for shelter.** The sick can afford to avail themselves of prompt and effective treatment. With the natural increase of the population, the demand for immigrant labour diminishes with a resultant decrease in the introduction of infection.

No opportunity was available for studying the village conditions in old rice areas where malaria has disappeared, as in the Gandia-Valencia district. The area of rice cultivation at La Cava in the Ebro Delta was visited. Here, probably largely as the result of the treatment campaign in force, the conditions seemed to be progressing rapidly towards the third phase. Valuable information was collected in this locality, which would appear to indicate the manner in which this phase develops.

At La Cava most of the farmers live in scattered and isolated dwellings in the midst of a sea of rice. These dwellings are mostly well built and in some cases of two storeys. Screening has been attempted in some instances to ameliorate the mosquito nuisance. These habitations are closely surrounded by the housing for domestic animals, which usually affords ideal conditions for the shelter of Anophelines. A few years ago malaria was very prevalent here but, probably as a result of the treatment campaign, it has diminished very considerably. The anophelism is so intense as to be almost unbelievable.

Enquiries made locally elicited some very interesting information, which would appear to indicate the method of spread of malarial infection in this area.

** Foot Note. In areas of rice cultivation, the animals' houses are grouped around human habitations, as this is often the only area not liable to periodical flooding during the agricultural operations.
In any of these isolated habitations, no malaria might occur for a year or more, in spite of the intense anophelism. On the other hand, if a human malaria carrier be introduced, the disease usually spreads through the entire household. The introduced carrier might be either an imported labourer, or a member of the family who had picked up the disease in the local village or some other place.

The infection appeared to remain localised and not to spread to neighbouring dwellings several hundred yards away. The following data about the mosquito population would appear to account for this localisation of infection:-

(a) Numerous suitable breeding places were present in the rice fields in the immediate vicinity of the dwellings.*

(b) The food supply is plentiful in the form of domestic animals.

(c) Suitable shelter is abundant and easily accessible, in the form of the dark and warm animal houses.

These conditions appear to form an ideal environment for the life cycle of the mosquito, and there would seem to be little need or cause for the insects to wander further afield.** Under these circumstances there seems little chance of the infection spreading to other distant houses. Even if an infected Anopheline should be carried or wander to another area, the influence of zoophilism, as well as the deterrent effect of screening, would greatly limit its chances of biting another human being.

---

* Foot Note. Dr. Gil Collado informed me that Anopheline larvae in this area were most plentiful near dwellings than further afield, an observation which supports the view that the insects do not wander far under conditions apparently so satisfactory for them.

** Foot Note. From the information available about Anophele

lipennis, the range of flight of this insect in any locality under normal conditions, would appear to be the distance to the nearest suitable food supply.
In this area, therefore, while the insect factor is eminently suited for the carriage of the disease, mosquitoes would appear to be merely a nuisance and not an actual danger until a human carrier is introduced. With the proper treatment of malarial cases and steps to prevent the introduction of gametocyte carriers, there would be a tendency for the disease to die out in such localities.

Under normal circumstances the change from Phase II to Phase III would only take place very gradually over a period of many years, during which the factors favouring the incidence of the disease diminished. In La Cava the arrival of this third stage is being accelerated by the treatment campaign.

---

* Foot Note. Routine examination of immigrant labour and treatment of infected cases is carried out in the Prat el Llobregat rice-area.

** Foot Note. This position with regard to malarial incidence appears to be very similar to that which forms the basis of the work in the new Italian colony at Macarese. At the latter place the new farm houses are collected in isolated centres and screened accommodation is provided both for the colonists and the immigrant labour. Treatment, both curative and prophylactic, is compulsory for all inhabitants of the area. The antilarval measures in force around the central village would tend to diminish the chances of acquiring an infection, when the inhabitants of the rural areas visit this place. Apart from these purely direct measures, the new colonists receive a subsidy to tide them over the first lean years. This work might be called an attempt to obtain rapidly a condition similar to Phase III of rice cultivation in Spain.
In some of the older rice-field areas cultivation is gradually spreading. Here no serious outbreaks of malaria have been reported, similar to those which marked the original undertaking in these localities. This is what might be expected, because the extension is largely carried out by local labour. In this way the dangers attendant upon aggregation of imported labour have been diminished, while the condition of the local people would be less suitable for the occurrence of an epidemic. This absence of serious outbreaks may, however, be partly attributable to the greater precautions (medical treatment and prophylaxis), which have been taken in recent years when any such outbreak was feared.

The conditions of rice cultivation in the Prat el Llobregat have a closer resemblance to Phase II than to Phase III. While at La Cava the cultivation is done by peasant farmers, in the "Prat" several large farmers have been licensed to undertake rice cultivation, and the work is mostly carried out by imported labour. This means that there is a continued influx of infected labour as in Phase II. To overcome this danger all labour working in rice fields must be medically examined. The result of the examination is entered on a card, which the labourer must produce on demand. If he be found to have malaria, he is given the choice between a full course of treatment and being returned to his home at the farmer's expense. All rice-field labourers must take prophylactic quinine. If one should contract malaria he receives free treatment and is paid while out of work.

**RICE CULTIVATION (PHASE IV)**

It seems probable that a fourth stage may occur in the history of rice cultivation. In this stage, while numerous Anophelines are present, malaria is absent ("Anophelism without malaria").
This condition has been explained as due either to
(i) The development of a larger and more robust type of insect,
the intestinal mucosa of which offers a greater resistance to the
invasion of the malaria parasite, or (ii) The comparatively rapid* appearance of a new zoophilic race of *A. maculipennis*, which seldom or never attacks man, if the blood of domestic animals be available. This race is supposed to have displaced an androphilic one.

A typical example of this phase would appear to occur at Massarosa in Tuscany. As the race of *A. maculipennis* from this area have proved normally susceptible to malarial infection, and is used extensively for experimental malarial transmission in connection with the treatment of general paralysis of the insane, no support is given to the first explanation.

As my only experience of such an area was the visit to Massarosa, so kindly arranged for me by Prof. Missiroli and Dr. Hackett, it is with considerable hesitancy that I put forward some suggestions in connection with this phenomenon.

While very much evidence has been produced to support the second hypothesis, this does not appear to me to have covered all the possibilities of the situation. It is quite possible that the changed conditions of the breeding places, which eventually develop in old-established areas of rice cultivation, and the greater facilities for feeding on domestic animals which have arisen, may make the region more suitable for a biologically different type of mosquito. It may also be that, in a comparatively short* period, there has been evolved a zoophilic race of *A. maculipennis*, or that such a race has replaced or displaced an androphilic one.

On the other hand, it is well known in malariology that changes in the nature of breeding places may result in the disappearance of one species of *Anopheline* with a marked increase of

* Foot Note. "Rapid" or "short" from the point of view of evolution.
another species. It therefore seems to me quite possible that the two types of insect reported may really be two different species, rather than two races of the same species with rapidly evolved bionomical differences.

Already the species, *A. maculipennis*, is being divided on morphological differences into several varieties by European entomologists. Even within the last few years *A. elutus* has been separated from the *maculipennis* group as a distinct species, both morphologically and bionomically. The *rossi-luillowi* group of Oriental Anophelines has been found, on recently discovered morphological differences, to be composed of many more species than those previously recognised, and these species vary considerably both in their bionomics and their potentialities for the carriage of malaria.

The evidence that such a zoophilic change occurs in the habits of the local Anophelines in some areas during a comparatively short time, cannot be neglected. It seems to me, however, that there is as yet insufficient evidence to prove that this change is not due to the replacement of one distinct species by another, rather than to a rapid change in the habits of individuals of the same species. It is easily conceivable that such a replacement of species might follow upon the changes in environment, which have occurred when a deserted and swampy area is converted into a populous and cultivated rice country.

If the phenomenon be due to a replacement of species or races, it might be possible to discover the biological or other conditions which govern this change, and thus hasten their production as a means of malarial control. On the other hand, if the change be due to the evolution of a new race, the process would be slower and more difficult to govern
or accelerate. Whatever the reasons of this change may be, its completion seems too slow and its causes too obscure, to make it form a practical method of malarial control in our present state of knowledge. Further research may, however, elucidate the mystery and devise a practical method of malarial control from the results obtained.

While this replacement of species or races of Anophe- line, may be the only cause of the disappearance of malaria in areas like Massarosa in Italy, there are possibly other contributary factors. The conditions in these old areas of rice cultivation may be merely an extension of the circumstances described in Phase III. The extensive quinine campaign which has been carried out in Italy during the last 30 years may have helped the fall in malarial incidence by causing a decrease in the number of malarial carriers in the Massarosa area. A decrease in the introduction of gametocyte carriers may also have helped in the matter, for apparently in some areas where such carriers were introduced after the War, malaria was again reported. I have not, however, sufficient information on these points to discuss them more fully and merely put them forward as suggestions.

Whether Phase IV exists in any of the areas of rice cultivation in Spain I am unable to say, but the conditions reported in the Gandia-Valencia area suggest that it may be present there.

CONCLUSIONS.

Three different phases of malarial incidence, and possibly a fourth, appear to occur in the history of rice cultivation in Spain.

The malarial incidence in the first phase seems to be caused mainly by aggregation of labour and its associated conditions.
The second phase appears to be the aftermath of the first. The malarial incidence is dependent upon factors connected with poor economic conditions and the gametocyte-carrier problem.

The low incidence recorded in the third stage follows as the result of the disappearance of many of the factors associated with poor economic conditions, and a diminution in the intensity of the gametocyte-carrier problem.

If a fourth stage be present in the older areas of rice cultivation in Spain, it may be associated with the gradual predominance of a species or race of Anopheline with marked zoo-philic habits.

The first step to be taken in controlling the malarial incidence in areas of rice cultivation in Spain would seem to be measures for mass treatment of the disease. These should always be reinforced by measures for the elimination or exclusion of gametocyte carriers.

Where possible the dangers associated with aggregation of labour should be counteracted and all possible steps taken to hasten the conditions of “bonification” found in the third stage of rice cultivation.

REFERENCES.


LEAGUE OF NATIONS.
CH/Malaria/203.
Geneva, March 28th, 1933.

HEALTH ORGANISATION

Malaria Commission.

The Medical Director has the honour to communicate here-with a Report on:

MACULIPENNIS RACES : THEIR MORPHOLOGICAL
AND BIOLOGICAL CHARACTERS.

by L.W. HACKETT, M.D.

This document has been prepared for the meeting of
the Study-Committee of the Malaria Commission.

Recent studies of the structural and biological charac-
ters of A. maculipennis enable us to divide the species into
at least four European races or subspecies. All four seem to
show genotypical variations and to differ from one another
almost as widely as they differ from A. elutus. The latter
might, therefore, be considered to be no more than a well-
marked subspecies of A. maculipennis. Descriptions published
by Herms and Frost indicate that the American maculipennis
also constitutes a distinct subspecies.

The following notes are based on careful observations
made by various authors in Holland, Germany, Denmark, Italy
and the Balkans. We have as yet insufficient knowledge of the
maculipennis races of the rest of continental Europe, of
Great Britain, Russia, and north Africa.

Differential characters of a morphological kind are
found to be most clearly marked in the egg, upon which our
present classification is based. The larva and the male imago
also possess structures which serve to identify certain
races but are inadequate for others. No structural differences have been found at all between the adult females of the several races. There are, however, such well-marked biological differences with respect to food and shelter preferences, climatic and hydrological limitations, domesticity, hibernation and sexual habits that we can no longer doubt that the character of the egg is a reliable and consistent guide to the race of the insect.

I. MORPHOLOGICAL CHARACTERS.

A. The Egg.

The eggs, according to the surface markings, fall naturally into two classes, the barred and the dappled types, which are undoubtedly genotypically distinct. These types were first described by Falleroni, who suspected that they represented racial or sub-specific differences, since all the eggs laid by a single female are of the same type. He named the barred-egg race *A. maculipennis* var. *messeae*, and the dappled-egg race *A. maculipennis* var. *labranchiae*. A practiced eye has no difficulty in distinguishing barred from dappled eggs.

The dark areas of the egg are not produced by surface pigment but by the brownish-black inner chorion showing through the more or less transparent portions of the enveloping membrane. This outer coat is thickly studded with irregular protuberances which appear like white incrustations reflecting the light with a brilliant silver sheen; but in certain spots the protuberances are reduced in size and at times almost suppressed, allowing the dark chorion to show through and produce the characteristic surface markings of the egg.
The common barred egg has two dark and well-defined transverse bands, one across each end just distal to the ends of the floats. The rest of the surface shows a rich pattern of irregular dark patches and oblique bars more or less accentuated, while the poles are also dark in colour. The whole surface may be so covered with dark areas that the egg appears almost completely black; or on the other hand the dark patches may be reduced to faint shadows, leaving the two fundamental black cross-bars in sharp contrast to the silver-grey field.

The dappled egg in contrast to the barred egg, shows a surface rather uniformly spotted with dark ovals and elongated black areas not sharply defined but shading smoothly into the light ground. These eggs may be very lightly dappled with a few small and indistinct spots so that they appear silver-grey under a hand-lens, or they may be heavily blotched so as almost to eliminate the points of light-reflection. In a few cases one or other of the elongated dark spots may extend clear across the egg, giving the impression of transverse bands, but these are usually unsymmetrical in position, irregular in width and occur in a few eggs only of a given oviposition, so that they cause no difficulty in classification.

The length of the float structure on either side of the egg is a character which also merits attention. It is longer in the barred eggs than in the dappled eggs, being usually more than 40% of the total length of the egg in the former, and less than 40% in the latter. Within each group, however, it may vary greatly.

There are three distinct varieties of the barred egg as illustrated in Figure 1.
Type a. is a uniformly black egg with large floats. It does not occur in pure culture or in conspicuous numbers anywhere, and it is not certain that it has been found north of the Alps. It occurs sporadically throughout Italy, but has not been reported in the Balkan peninsula. In size, shape and float-structure, it is exactly like the common barred egg, with which it is connected in nature by a complete series of intermediate gradations, and it may be merely a modification without genotypical character. It has not been given a special name.

Type b. is the common barred egg of the race A. maculipennis messeae Falleroni, as described at the beginning of the section. This is the prevailing type throughout the European range of A. maculipennis except in the extreme south (Calabria, Sicily) where it becomes rare. It is often found in pure culture over great areas, especially, as we shall later show, in continental river valleys.

Type c. is the simple banded or striped egg which has as its only markings on a silver-grey ground two heavy transverse bands, together with a darkening around the poles. In almost every deposition, however, eggs occur with faint shadows in the interbandal zone and a pure example of the two-striped egg is not easy to find. There are a number of intermediate types linking this with the common barred egg of messeae, but on the other hand we know of several rather circumscribed regions (Schito, Fucino) where it is the only egg to be found, and unlike the black egg, it occurs throughout the entire range of A. maculipennis thus far surveyed, being found both in Sweden and in Sicily. There can be no objection in assuming that this ubiquitous form was the one described by Heigen, and we have called this race A. maculipennis maculipennis (typicus).
Of the dappled egg there are at least two varieties.

**Type d.** is a somewhat smaller egg than the others, slightly shorter and more slender, with a float structure varying considerably in length in different regions and probably also with the season, but always smaller than that of *messuea*. The more important identifying characters are, however, the brownish-grey tone of the egg as contrasted with the silver and at times almost blue-grey of the other dappled variety, and the clear transparent membrane covering the air chambers of the floats. Swellengrebel first pointed out that the eggs of the "short-winged" sub-species in Holland had smooth walled floats and this distinguishes them, as far as we know, from the eggs of all the other members of the *maculipennis* group. The latter show finely wrinkled float walls, with close-set minute cross lines or corrugations, rendering them non-transparent. These fine cross lines, which seem rather to be folds than thickenings in the membrane, are usually not straight but are slightly sinuous and often branched. (See Figure 2.)

The egg is usually sombre in appearance owing to the size and number of the dark spots, and one or another of these may be considerably elongated so as to extend right across the egg, giving the impression of irregularly placed transverse bands, as has been previously mentioned.

This egg, wherever it is found, seems to be identical with that of the species discovered by van Thiel which he has named *a. maculipennis* var. *atroparvus*. 
Type e. This egg, which we have sometimes referred to as the "dappled-grey" to distinguish it from the "dappled-brown" egg of type d., is a large, thick egg with very small floats which, in the southernmost range of the subspecies, may be only 20% of the total egg length. The egg may appear light or dark grey, depending on the amount of spotting, but in contrast with the atroparvus egg there is very little tendency to false banding. The eggs can be distinguished from those of atroparvus by color and form alone, but the principal point of differentiation is the roughness of the float wall as compared with the smooth membrane of the atroparvus float, which appears like celophane under the microscope. Owing to the small and narrow float structure, the dappled-grey eggs are often found on the surface of water lying side by side in long rows. The eggs with larger and more protuberant floats are apt to be disposed in triangular or radiating patterns. Falleroni used the name labranchiae to include all the dappled egg mosquitoes, but this name is now confined to the race laying eggs of type e.

Type f. shown in Figure 1. is the egg of A.elutus, very closely allied to labranchiae. This egg has the same form and dimensions but has a uniformly silver-grey surface, composed of thickset, light-reflecting incrustations, outlined in black where the chorion shows through between them. The elutus egg has no floats at all in summer time but may show rudimentary floats in autumn and winter. It has instead a very wide fringe which is continuous round the entire egg.

From a description furnished by Frost, the maculipennis egg of California has a uniformly grey surface, without design, and with permanent but rather small floats.
The situation with regard to classification of *maculipennis* eggs may be represented by the following diagram.

```
A. maculipennis
   | barred eggs
   |   (black) ........ type a.
   |   | messeae ........ " b.
   |   | typicus .......... " c.
   | dappled eggs
   |   | atroparus ........ " d.
   |   | labranchiae .... " e.
```

Of these varieties it is very probable that at least four are genotypes, although the division between *messeae* and *typicus* and between *atroparus* and *labranchiae* is perhaps of a less definite order than that between the barred and the dappled types. The black egg is possibly only a modification or an extreme form of *messeae*, but of this we know very little.

B. The Larva.

De Buck, Schoute and Swellengrebel (1932) found that the antepalalte hair (No. 2, according to Martini) of the fourth and fifth abdominal segments has more branches in the "long-winged" race (*messeae*) than in the "short-winged" (*atroparus*). Adding together the branches of the antepalalte hairs on the same side of the fourth and fifth segments, they state that the number is more than seven (average 9.3) for the "long-wings", and less than seven (average 5.9) for *atroparus*. Since the hairs on one side of the larva may differ in this respect from those on the other, each side of the larva is considered a separate "case" in computing the combined number of branches. La Fice, studying the same hair in *labranchiae*, *messeae* and *maculipennis* in Italy, found the total number of branches as computed above to be on the average less than five for *labranchiae* and more than nine for *messeae*. *maculipennis typicus* is found to hold an intermediate position. This character, therefore, serves rather clearly to divide the barred egg group on the one hand from the
dappled egg group on the other but is untrustworthy as a means of separating members of each group.

La Face (1929) also pointed out that hair No.1 (Martini) on the second abdominal segment is a true palmate structure in labranchiae and a simple branched hair in messee. (See Figure 3). Atroparvus and maculipennis typicus show intermediate forms. This character is of value in separating labranchiae from the barred egg group in the many southern regions where atroparvus does not exist. It is evident, however, that none of these characters is sufficient for the complete identification of a given larva.

C. Male Terminalia.

De Buck, Schoute and Swellengrebel (1930-1932) described certain differences in the male hypopygia of the "long-winged" and "short-winged" races of Holland. The dorsal (external) spine of the claspette, (See Figure 4) whether single or double, was sharp-pointed in atroparvus (99.7% of the mosquitoes caught in nature), whereas the same spine in the larger mosquito was blunt in 40% of the cases. Martini, studying males caught in zones where the different races are known to exist in almost pure culture, describes this spine as practically always blunt in typicus, as prevailing blunt in typicus, blunt in messee, and as sharp-pointed in labranchiae and atroparvus. La Face, studying the characters of the hypopygium in males bred in the laboratory from three types of egg, finds that the external spine of the dorsal lobe of the claspette is definitely short and blunt in typicus, is still blunt though somewhat longer on the average in messee, and is uniformly sharp-pointed in labranchiae. This spine is also shown as sharp-pointed in van Thiel's figures of atroparvus.

It is probable that the Dutch authors found the spine blunt in only 40% of the "long-wings" because they were not dealing with a pure race but with a mixture of messee and atroparvus,
which they were unable at that time to separate because the egg and float characters were unknown.

We have here probably a means of distinguishing between the barred egg group on the one hand and the dappled egg group on the other, but not between members of the same group.

D. Female Imago.

Except in the special case of elutus no morphological characters have as yet been found which might serve to classify the adult female. A difference in size and coloration independent of environment was noted by the Dutch authors between the "long-wings" and "short-wings" of Medemblick and Leiden respectively, and this, in fact, led to the separation of maculipennis into two varieties. These differences are without doubt genotypical but can be expressed only in statistical form, and hence have no value for the identification of the individual mosquito. Furthermore, the effect of temperature and nutrition on the larval stage of the mosquito has such a decisive influence on the size of the imago that they tend to obscure the less important genetic differences, so that comparisons cannot rightly be made between specimens taken in different places, different latitudes, different years or even in different seasons. It is a fact that the largest maculipennis (typicus) of south Italy is smaller than the smallest atropervas of Holland. Hence somatic measurements, number of teeth, etc. have little or no taxonomic value.

II. BIOLOGICAL CHARACTERS.

Space will permit of only a brief mention of the differences in behaviour and other biological characters observed in the adults of the above-mentioned races.

A. Adaption to Climate.

Mesops has a very wide range to the north and south of the Alps. It becomes less frequent south of Naples and only rare
individuals are found in southernmost Italy and Sicily. We have no information as to the races of north Africa.

The nearly related typicus has an even wider range and has been found in every region where it has been sought, including such widely separated regions as Norway and Greece.

Atroparvus is a distinctly northern type and has been found in Italy only in the valley of the Po. It probably also occurs in northern Spain.

Labranchiae appears to be a subtropical form and has been definitely identified only in Italy south of Rome. It does not occur in the Balkan peninsula. Occasionally very light specimens of dappled eggs with small floats have been reported from northern Europe, without indication however as to whether the walls were smooth or rough. We cannot yet be sure that they are not modifications of atroparvus eggs.

B. Adaption to Hydrological Conditions.

Messeae is found in the great river valleys (above sea level), in inland plains and marshes where the water is clean and sweet, and in the vicinity of fresh water lakes, great and small. An exception is found in Italy in the marshes between Viareggio and Massarosa, which are near the sea but where automatic tide gates (installed a century ago) keep the water from becoming brackish.

Typicus seems to be particularly the mosquito of fresh spring water and of cold water in general, but there are exceptions. It occurs in the most northern latitudes (Oslo), in areas of a certain altitude (Black Forest, Fucino) and in connection with the sources of fresh water streams (Orte di Schito, Mundenhof, Wohldorf, etc.) This is a ubiquitous mosquito and the hydrological limits of its breeding are not yet well defined.

Atroparvus is the characteristic mosquito of low lands reclaimed from the sea. In Holland, Germany (East Friesland) and Italy (Po delta) it is found in areas below sea level which are
drained by pumps. It also occurs on the islands of the North Sea and is present, though not in pure culture, in inland localities with saline springs, lakes or marshes. It seems to be present in polluted waters in relatively greater numbers than messaeae. In short, it is the mosquito of brackish waters in northern Europe but in Italy it occurs in the relatively sweet irrigation water of the Po delta, which is, however, beyond doubt affected by the comparatively superficial peat beds.

Labranchiae is the brackish water mosquito of Italy, together with A. elutus. These races in turn invade fresh as well as brackish waters in the southernmost portion of their ranges. Thus labranchiae is found as practically the only maculipennis of Sicily and Calabria where messaeae can no longer adapt itself to the warmer conditions; while elutus breeds in sweet water in Palestine where not even labranchiae seems to be able to maintain itself, and hence where no member of the maculipennis group proper is found.

C. Sexual behavior.

It appears that the dappled egg races will mate without difficulty in confined spaces (according to Roubaud in 1/20 m³), whereas the barred egg races require the nuptial flight and have not yet been observed to copulate in captivity. The Dutch authors and Roubaud both report the fecundation of barred egg females by atroparvus males. The resulting generation, of mixed breed, mated in captivity, but the second mixed generation obtained from this failed to cross breed (Roubaud).

D. Hibernation*

The two principal maculipennis races of northern Europe behave differently with regard to hibernation. The dappled egg

race (atroparvus) gradually stops all ovarian activity in August but continues in general to frequent warm stables and bite throughout the winter. The barred egg group (messeae) rather suddenly ceases egg-laying about a month later and migrates into cold shelters for complete hibernation. There is no difference in fat content between the races on chemical examination. One stays in warmer places but replaces metabolised fat by occasional bloodmeals. During the winter all the insects are in the stables, if the only race present is atroparvus, all in the cellars or attics (cold unoccupied rooms) if messeae. With a mixed population, segregation by races occurs. In the lower Rhine valley, the barred egg race emigrates to higher land in winter, but no such migration has been noted with the dappled egg race. Placed in a warm environment, both races will suck blood in winter, showing that the need for food depends on the physical conditions (microclimate) of the place chosen for overwintering and not vice versa. In one area (Twixlum) the dappled egg race was found in complete hibernation in attics, showing that the choice of complete or of semihibernation does not seem to rest on a physiological basis and to be a racial character in itself, but that the races seek different microclimates for the winter, perhaps even without regard for temperature, which may be an accidental accompaniment of other conditions sought. If this is low they hibernate, if high they feed all the winter. The dappled egg race seems not to seek higher places and to be adapted to warmer or moister conditions. Temperature does not play a decisive role in concluding hibernation which depends also on other conditions. The dappled egg race gradually resumes egg-laying in March and the barred egg race a month later, rather suddenly. But this is not a
racial difference; it depends on the kind of hibernation. Those in semi-hibernation awake first. The only difference between the races then is one of choice of microclimate in autumn - one chooses stables, the other leaves them.

As one proceeds from north to south toward warmer winter temperatures this distinction tends to fade out, so that south of Naples even typicus and messeae hibernate incompletely. This confirms Martini's suggestion that the mode of hibernation depends not on a deep-seated instinct but on the conditions obtaining in the place which the insect chooses to pass the winter.

One curious fact is that, although in winter ovarian activity is suspended in nature in all races of maculipennis, yet in captivity, if kept at 20°C., 50 to 60% of atropurvus and labranchiae will lay eggs even in winter after a blood meal, whereas messeae and typicus are almost entirely refractory under the same condition.

De Buck, Schouten and Swellengrebel state that in Holland it is difficult to induce "long-wings" (messeae) to feed and if they do so the blood forms a contracted clot at the lower end of the stomach with clear transparent serum at the upper end, and the insect will not feed again. "Short-wings" (atropurvus) feed readily and the blood fills the stomach and is easily digested. This corresponds to observations by Martini that atropurvus, though it usually feeds throughout the winter, is sometimes found in complete hibernation in empty stables, whereas messeae always hibernates
completely in cold climates and never frequents occupied stables, where it would be forced by an increased metabolism to feed from time to time.

E. Association with Man.

All races of *maculipennis* frequent stables more than bedrooms, but, whereas the barbed egg races and *atroparvus* are often deviated entirely toward domestic animals and bite man only under exceptional circumstances and very irregularly, *labranchiae* and *elutus* bite man and animals indifferently, and 10 to 20% of the total number resting in buildings is to be found in dwellings. Of all the races *typicus* is least associated with man and even in captivity only about 70% of famished females can be persuaded to take human blood. In this case it appears as though there were a rather strong preference for the blood of lower animals.
Both *maseae* and *atroparvus* are ordinarily cattle feeders but both, on occasion, will enter human habitations to bite. At Muntua, a town with relatively few stabled animals, surrounded by marshes which are prolific breeding places, a certain (if comparatively small) number of *messeae* enter bedrooms and feed upon human beings. In this way they succeed in maintaining endemic malaria, but at such a low level of intensity that it cannot be considered of public health importance. Before storms and under other special weather conditions, both Martini and Hackett have noted *messeae* entering houses and biting human beings.

*Atroparvus*, although its food habits are much like those of *meseae*, is a more dangerous mosquito because, after the suspension of ovarian activity in the autumn, it does not go into complete hibernation like *messeae*, but continues to visit occupied stables and not infrequently houses. Being relieved of the necessity of issuing forth to lay eggs, it may remain for long periods indoors though it may possibly change its quarters during mild spells in the winter.

*Labranchiae* frequents houses as well as stables under all conditions and in spite of the number and accessibility of domestic animals. Staining experiments have repeatedly shown that it has no food preferences but will wander back and forth between bedroom and stable on successive nights.

III. **MACULIPENNIS RACES AND MALARIA.**

Very little is known as to the causes of the intense malaria of a century ago in northern Europe, or of its rapid decline. It can only be stated as a fact that at the present time intense endemic malaria is only found in connection with *A. maculipennis labranchiae* and *A. elutus* or some entirely different
species of anopheline. The early confusion between the two
dappled egg races (atroparvus and labranchiae) created the
erroneous impression that the malaria of north Holland and
north Germany was caused by the same mosquito responsible for
the intense malaria of Sardinia and southern Italy. It is now
known that neither atroparvus nor messeae are dangerous mosqui-
toes. Their association with man is not usually sufficiently
frequent or regular to maintain malaria; but under special con-
ditions, due to an influx of gametocyte carriers, the production
of anophelines in extraordinary numbers, lack of domestic ani-
mals, or a combination of other environmental factors, either
race may for a short time cause sharp epidemics of malaria or
maintain a very mild malaria endemic at a low level of intensity,

It is an interesting fact in this connection that only
barred-egg maculipennis occur in the Balkans, even in the most
malarious spots. Recent dissections on a large scale by Barber
and Shannon have shown, however, that the principal carriers
in this region are A. elutus and A. superstictus, which at the
height of the annual epidemic in northern Greece often show an
infection-rate more than ten times as great as A. maculipennis.
Figure 3. Character of hair, No. 1 (median) of the second abdominal segment of the Maculigera larvae.

Figure 4. Types of A. maculigera and A. elitus.

A. v. maculigera L. var. A. v. maculigera orientalis.
B. v. maculigera australis.
C. v. maculigera maculigera.
D. v. maculigera japonica.
spine, which may be double.

A. maculipennis messee.

A. maculipennis atropavus.