Section of Tropical Diseases and Parasitology.

President—Professor J. GORDON THOMSON, M.B.

[*January* 7, 1932.]

Synthetic Anti-Malarial Preparations.

A Discussion of the Various Steps which led to the Synthesis and Discovery of "Plasmoquine," and a Brief Account of its Use in Tropical Medicine.

By Professor Dr. W. SCHULEMANN,

Elberfeld, Germany.

WHEN my colleagues and I took up the problem of the synthesis of anti-malarial preparations in the Bayer-Meister-Lucius Research Laboratories, we had solid foundations to build upon—foundations laid piece by piece during several decades by other workers.

In 1880 Laveran discovered the malarial parasite, and in 1891 Grassi and Feletti found in birds a parasite similar to that of human malaria. In 1895, Ross, stimulated and directed by Manson, discovered the rôle played by the mosquito in transmitting the disease.

How bird malaria might be used for the study of malarial treatment in man was investigated by Kopanaris and the brothers Sergent, but it was not till 1924 that a satisfactory technique was evolved by our colleague Dr. Roehl, whose recent early death we most deeply regret.

Roehl worked out a method of using canaries for experiments on lines closely approaching the conditions of practical therapy, so that it was possible to try out and assess in the laboratory many groups of drugs. This method was fully described by Roehl himself in Düsseldorf in 1926. Wagner Jauregg began the treatment of general paralysis by artificially infecting the patient with malarial 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 of anti-malarial drugs could be carried out on the human subject even in this northern climate.

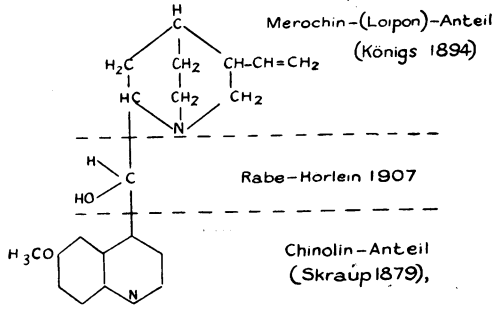
The therapeutic action of chinchona bark in malaria was recognized by the natives of Peru centuries ago, and in 1638 the physician de Vega used the bark to treat the wife of the Spanish ambassador, the Countess of Chinchon, who was suffering from malaria, so that its properties became known in Europe.

In 1820 Pelletier and Caventou prepared quinine from the chinchona bark, and the work of many investigators during the next half century finally enabled its chemical constitution to be formulated.

Diagram I shows the formula which is now ascribed to quinine.

Attached to a quinoline ring, which was identified by Skraup in 1879, there is a basic heterocyclic group (meroquin or loipon group), which Königs described

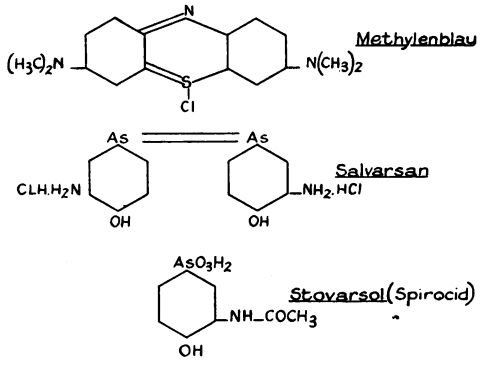
DIAGRAM I.



in 1894. These two parts are united by a bond. On a suggestion from Hörlein, this formula, now generally accepted, was laid down by Rabe in 1907.

An extensive series of experiments was undertaken with the object of discovering other combinations which might be effective in malaria, but the only ones with any definite action were found to be methylene blue and salvarsan. The constitution of these is shown in Diagram II.

DIAGRAM II.



Ehrlich and Guttmann discovered the action of methylene blue in 1891, and Werner, in 1910, that of salvarsan.

Later, Marchoux tested the anti-malarial action of stovarsol (spirocid), a drug prepared by Fourneau from an intermediary product of salvarsan.

In connection with the constitution of quinine, an extensive series of synthetic products was prepared by many investigators with a view to finding an effective compound against malaria. It was generally assumed that an anti-malarial drug must contain a quinoline nucleus, with an aliphatic basic group bound by a carbon bond to the fourth position of quinoline. In spite of much excellent synthesis, however, the desired goal was not reached.

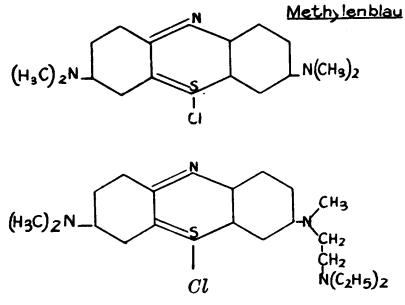
My two colleagues Schönhöfer and Wingler, together with myself, based our experiments on methylene blue. In one of the aromatic amino groups of methylene

blue we substituted an aliphatic basic group in place of an alkyl radical (one of the methyl groups).

This is shown on Diagram III.

Roehl found this compound to be effective in bird malaria, but it had the definitive characteristics of a dye-stuff. Therefore we transferred the experience we had gained from methylene blue to the quinoline group. Contrary to the earlier views of other authors, we did not unite the basic aliphatic radical by a carbon bond

DIAGRAM III.

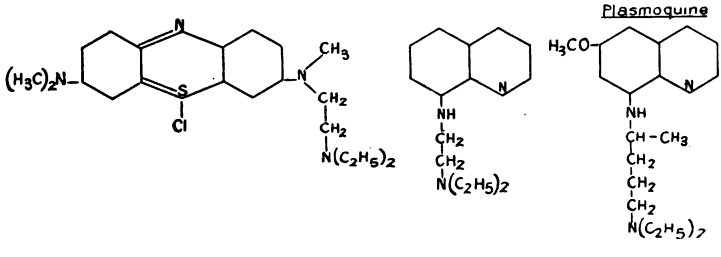


in the fourth position, but with a nitrogen bond to the quinoline nucleus. The first compound of this series which we prepared was a derivative of eight (8)—amino-quinoline. Roehl found this to have a marked therapeutic action on proteosoma infections in canaries. The hydrochloride of this compound had a yellow colour, but without the properties of a dye.

Diagram IV shows the formula.

This new knowledge formed the basis for further work, and by full and free collaboration between chemists and physicians these beginnings were developed.

DIAGRAM IV.



We changed the position of the amino groups in the quinoline ring, introduced every conceivable substitute in the quinoline nucleus in addition to the amino group, and also used many other heterocyclic rings. Furthermore, we varied the side chains and finally the basic aliphatic group.

Diagram V shows a few examples of variations of the side chains.

The length of the carbon chains was altered, and it was made into branched chains in many different ways. We interrupted these chains with single or multiple ethereal oxygen and sulphur atoms and intermediate amino groups. We introduced free or esterified hydroxyl groups into the carbon chains and made many further variations.

The action of all these compounds was tested in animal experiments by Roehl, and from among them we chose plasmoquine for practical clinical investigation.

DIAGRAM V.

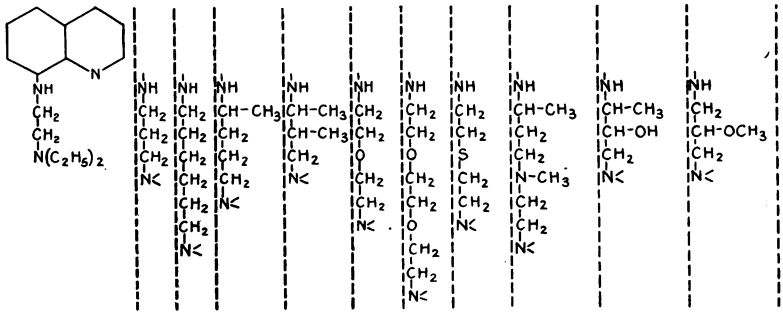
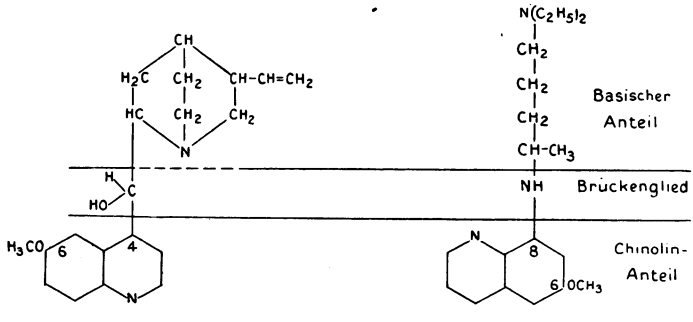


Diagram VI will enable you to compare the constitutional formulæ of quinine and plasmoquine, and to make the comparison clearer the quinoline ring in the plasmoquine formula is placed upside down.

The basis of quinine, as of plasmoquine, is six (6) methoxy-quinoline. In quinine there is a carbon bond in the fourth position, while in plasmoquine an amino group is the bond in the eighth position. In both quinine and plasmoquine the attached side chain is a basic aliphatic residue. In quinine this residue is a tertiary amine, in which the aliphatic nitrogen is joined in a heterocyclic ring. The basic fraction of plasmoquine contains a tertiary nitrogen without a ring. A further interesting fact is that the gross formula and the molecular weights of both compounds differ very little.

DIAGRAM VI.



$C_{20}\ H_{24}\ O_2\ N_2$
Mol. Gew. 324

$C_{19}\ H_{29}\ ON_3$
Mol. Gew. 315

On the basis of the toxicological and chemo-therapeutic properties we selected plasmoquine in 1925. Sioli found it therapeutically effective in malaria artificially inoculated for general paralysis.

In naturally acquired human malaria the action of plasmoquine was demonstrated by Roehl, who was also the first to discover that plasmoquine destroyed the gametocytes of *Plasmodium falciparum* within a few days, a result which cannot be achieved by quinine. This was a surprising fact; it meant that our work had not

produced a substitute for quinine. Plasmoquine is a synthetic anti-malarial drug, but in its therapeutic properties it differs from quinine.

The first clinical experiments with plasmoquine on a large scale were carried out by Mühlens and his co-workers, by Manson-Bahr and many others. Following this pioneer work, and with the assistance of many investigators, the development of plasmoquine therapy proceeded apace.

But I neither wish to detail here a bibliography of all the papers which have been published on plasmoquine, nor, on the other hand, do I intend to speak about the therapeutic value of plasmoquine in malaria. I propose to deal in this paper only with those facts which, in my opinion, are of importance in indicating the lines of further investigation.

It is to-day well established that the three types of *Plasmodium*, *Plasmodium vivax*, *Plasmodium malariæ* and *Plasmodium falciparum*, react to drugs in a definitely different way, although the reactions are not fundamentally different.

We know, for example, that arsenic compounds have a distinct therapeutic action on *Plasmodium vivax*, but they have, on the other hand, little effect on *Plasmodium falciparum*. Again, *Plasmodium malariæ* is influenced therapeutically to a certain extent by methylene blue, while on *Plasmodium vivax* this effect is small and on *Plasmodium falciparum* it is non-existent. Quinine does not produce the same effect on all three species; it is most potent on the parasites of benign tertian malaria, less active on those of quartan, and least of all on those of subtertian malaria. This is particularly the case in acute malarial attacks.

With regard to relapses, particularly delayed relapses, quinine has less effect on *Plasmodium vivax* than on *Plasmodium falciparum*, that is to say, the relapse-rate following quinine therapy is considerably higher in tertian malaria than in subtertian malaria. These variations in the action of quinine against different types of *Plasmodium* would indicate that the various stages of development of the parasites react to drugs in different ways.

The investigations carried out with plasmoquine confirmed this. The work of medical officers in British India, among whom I would particularly mention Sinton, Knowles, Wallace and Manifold, demonstrated that with plasmoquine therapy, or with plasmoquine combined with quinine, it was possible to reduce the relapse-rate of tertian malaria from fifty (50) per cent. (the usual rate associated with quinine administration) to between two and five per cent. (2-5%).

In subtertian malaria the results do not appear to have been quite so satisfactory, although here also there seems to have been a certain reduction in the number of relapses following plasmoquine therapy.

On the other hand, extremely interesting results were obtained in subtertian malaria with regard to the action of plasmoquine on the gametocytes. Observations on this action have, however, not established any definite facts so far. All we can definitely assert is that in regard to *Plasmodium vivax* plasmoquine acts on the schizonts as well as on the gametocytes. We cannot as yet form a definite opinion on the action of plasmoquine on the gametocytes of tertian malaria, and we await with interest the outcome of the investigations of the Institute in Kuala Lumpur, in the hands of Kingsbury and Amies.

At the moment it is only in subtertian malaria that we can assess plasmoquine with any degree of accuracy. Quinine acts effectively on the schizonts of *Plasmodium falciparum* and allays the acute clinical symptoms, but it cannot inhibit the development of gametocytes—in fact, it appears to stimulate this phase. Plasmoquine, on the contrary, has practically no effect on the subtertian schizonts, but it definitely does destroy gametocytes in a few days. The early view that plasmoquine could inhibit the formation of gametocytes has not been confirmed. On the other hand, the work of Barber, Komp and Newman, Withmore, Roberts and Jantzen, in the hospitals under the direction of Deeks of the United Fruit

Company in Central America, definitely proved that even minimal doses of plasmoquine which were too small to cause the subtertian gametocytes to disappear, sufficed nevertheless to render the gametocytes incapable of infecting the anopheles.

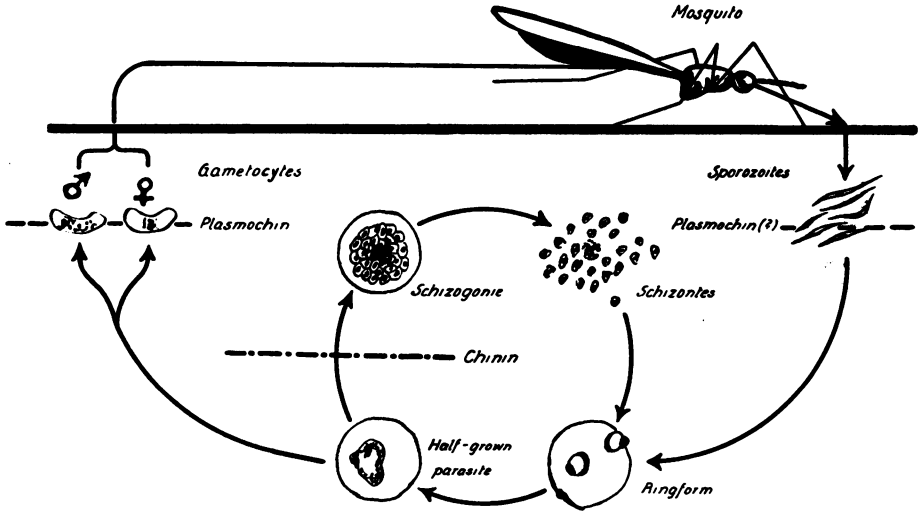
Here, then, for the first time, it appeared possible effectively to break the cycle of infection—man—mosquito—man. Naturally, this gave rise to the consideration of malaria prevention by means of plasmoquine administration—where experience in former years had shown that quinine alone was not sufficient. If it were possible to prevent the anopheles from becoming infected by giving a drug to the human host, then, in theory at least, malaria might be stamped out.

Barber wrote to me recently to say that in West Africa he had succeeded in putting these theoretical conclusions into practice. He intends to publish his results in the near future.

In practice, however, one must never lose sight of the fact that it will be possible only in exceptional cases to treat the population of a malarial district so thoroughly and regularly by means of drugs that every gametocyte-carrier can be included, and so render it impossible for any mosquito to become infected. I am in entire agreement with Sir Malcolm Watson that the discovery of plasmoquine has in no way rendered superfluous the usual sanitation methods directed towards the extermination of the mosquito. At the same time the advent of plasmoquine will provide a new and important weapon allied with sanitation in the fight against malaria.

In considering the places of development of the malaria parasite as I have portrayed them in diagram VII—and I confine myself here purposely to subtertian

DIAGRAM VII.



Subtertian Malaria

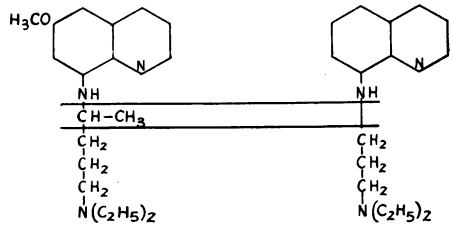
malaria—we see first, a half-grown parasite arising from the schizont. This parasite can continue its development in two directions—the growing parasite can either turn to schizogony, against which quinine is active and plasmoquine inactive, or else gametocytes may be formed, against which plasmoquine alone is effective while quinine is not. As I have set out already, we can assume with certainty, in

subtertian malaria, that quinine breaks the cycle of development at the · — · — · — · line, while the point of attack of plasmoquine is marked in — — — —

There are however two points in this scheme of development which have hitherto escaped attention. The first is that we know neither which phase of the parasite gives rise to relapses or recurrences, nor what drug affects this phase.

A second point is the question whether there is a drug that affects the phase of development occurring between the sporozoite inoculated into the human body by the mosquito, and the ring form. If so, what drug? It is to this problem that Warrington, York and Macfie have turned their attention; they showed that in transmitting malaria from one general paralytic patient to another through the medium of mosquitoes it was not possible to obtain a prophylactic action with quinine, even when administered in maximal daily doses. Thus these observers were able to show that quinine has no causal prophylactic action in malaria. Thus quinine suppresses the symptoms of infection, but does not prevent infection. The latest work of James shows that plasmoquine on the other hand in doses of 0·06 gramme daily for six days acts as a causal prophylactic or, to adopt James's term plasmoquine may be considered as a "chemoprophylactic." It is therefore necessary to indicate the second point of action for plasmoquine in our diagram, and this I have shown by the second — — — — — line. But we must wait until this work has been completed before the significance of these findings can be correctly assessed. Yet the scientific importance of this work is already definitely established, since it has drawn our attention to many new aspects of the problem which reveal the way to discover new drugs, differing both from quinine and from plasmoquine, which might attack the cycle of development of the malaria parasites at other points.

DIAGRAM VIII.



Plasmoquine Fourneau 710

Recent work in our laboratories in Elberfeld goes to show that these considerations are following the right lines. I shall say a few words regarding them, but first I shall cite briefly what has already been done by other investigators in this field. In America, Hegener, Shaw and Manwell, in England, Barger and Robinson, in conjunction with Macfie and Keilin, have already published their results. In France, Fourneau published in 1931 a paper on a synthetic anti-malarial compound prepared by himself, which he called Fourneau 710 and submitted to clinical trial. Diagram VIII shows the constitutional formula of this body alongside that of plasmoquine; the difference between the two lies in a shortening of the carbon chain.

The experiments of Bramachari in Calcutta have so far given no practical results, while Collier, Warstadt and Krause have not yet communicated the outcome of their investigations.

According to a short communication in the *Sowjetskaya Pharmazia* (March, 1931) the Russians appear to have produced anti-malarial combinations of quinoline which are at present undergoing clinical tests.

In our laboratories in Elberfeld, Mietzsch and Mauss have succeeded in forming new compounds while developing the work on plasmoquine by formulating another heterocyclic ring system; these compounds have been tested in animal experiments

by Kikuth, the head of the Bayer-Meister-Lucius Chemotherapeutic Institute in Elberfeld. Kikuth employed a new experimental method which he evolved and will publish later. By his method he has determined, not only that the new compounds were effective against malaria, but also that this action differed in principle from that of plasmoquine. Kikuth is of opinion that our new product is likely to be effective against the schizonts in the same way as quinine is. And since Sioli showed that the product was effective in inoculated malaria in general paralysis, we have sent supplies of it to a number of investigators in the tropics and sub-tropics for practical therapeutic tests. Our expectations have been realized. The compound acts on the schizonts of malaria and has no effect on the gametocytes. We have no data as yet regarding its effect as a prophylactic.

The mode of action of this new compound again proves that anti-malarial drugs possess specific properties. It may be—and the future alone will show—that the new compound has other potentialities in its action. It seems to offer advantages in dosage and duration of treatment, and also it appears capable of materially reducing the number of relapses after treatment. I have tried to give in general terms the first impressions we have formed regarding the action of this compound in practice. A full account of the details must be reserved for the publications of those workers who are now and will be in future investigating its effects.

I have endeavoured to show how each step has been built up on the results of work done in the past. Based on this ground-work, we may perhaps have added some small contribution to the edifice. Whether the next step will be furnished by other workers or by ourselves is of little moment—all that matters is the advancement of science and the help that can be given to the sick.

Discussion.—Colonel S. P. JAMES: Plasmoquine is the first effective anti-malarial drug that has been prepared synthetically in the laboratory, and Professor Schulemann's decision to come to England to explain in detail the history of its discovery and the exact nature of its chemical constitution was a friendly gesture which I am sure will be fully appreciated in this country. The discovery is noteworthy, not only because plasmoquine is a potent anti-malarial weapon, but because observation of the results of its action on the malaria parasite led to an important change in ideas and aims concerning the chemotherapy of malaria. Formerly it was generally believed that malarial chemotherapy comprised only the single problem of finding a drug which acts more effectively than quinine in curing attacks of the disease, but now it is realized, as Professor Schulemann has explained so clearly in his paper, that the problem is not a single one but includes four items of research, each of which must be considered separately. There is first the problem of the sporozoites; secondly, that of the asexual parasites; thirdly, that of the sexual forms, and fourthly, that of the forms responsible for relapses. Perhaps in the future a drug may be found which will act equally well on all those stages, but at present the conclusion to be drawn from a knowledge of the action of plasmoquine is that a different drug must be used for each stage. For destroying the sporozoite stage plasmoquine is as yet the only known drug with an effective action, but even plasmoquine is not entirely satisfactory for the purpose because, according to our latest experiments, it seems probable that, to secure complete protection from infection in an intensely malarious place, one would have to take more than four centigrammes daily, and even four centigrammes is a dose which might produce toxic symptoms if taken daily over a long period. For dealing with the asexual stage, quinine is already available, and perhaps for that purpose a better product would be hard to find. On the sexual stage plasmoquine acts excellently and in doses smaller than are necessary for destroying the sporozoite stage. Lastly, for the stage that is responsible for relapses there is as yet no known drug which acts effectively. Professor Schulemann has mentioned some reports in which it is stated that the relapse rate after a course of plasmoquine is less than the relapse rate after a course of quinine. I cannot help thinking that the observations recorded in those reports may be open to a different interpretation, and that, at any rate in malignant tertian fever, plasmoquine is not more effective in preventing relapses than is quinine. The diagram [shown] of the clinical course of five cases of malignant tertian malaria treated at Horton is of interest in this connection.

You will see that in all these cases a course of plasmoquine, either when given alone or when combined with quinine, failed to prevent relapses. The cases shown on the diagram are of a severe type, but they exemplify what seems to us at Horton to be a chief difficulty in appraising the therapeutic value of new remedies. The difficulty is that each species of the malaria parasite includes several different varieties or strains, of which some must be regarded as being quite virulent, others quite mild. This is particularly obvious as regards different strains of *P. falciparum*. At Horton we have worked with seven different strains of *P. falciparum*, of which two originated in India, one in W. Africa, one in Sardinia and three in Rome. Quinine in small doses sufficed to cure all the cases infected with the Indian strains, but the cases infected with the Rome and Sardinian strains were very refractory to this drug even in large doses and on several occasions we have been quite at a loss to know how to cure them. The diagram illustrates five cases of that type. The experience shows that before venturing to say that a new drug possesses remarkable curative properties one must first ascertain that the cases on which it is being tried were not infected with a strain which could be cured as easily by moderate doses of quinine.

In conclusion one might say that for malarial chemoprophylaxis the primary need at present is a drug that has the same action as plasmoquine but is somewhat less toxic, and for malarial chemotherapy a drug which when taken in therapeutic doses for a short period will effectively prevent relapses. I gather from what Professor Schulemann has said to-night that one or more new preparations which he hopes will fulfil those requirements may soon be available. We shall look forward with the greatest interest to that prospect, and in the meantime I should like again to express my admiration to him and to his co-workers for the remarkable advances in the chemotherapy of malaria which they have already made.

Sir MALCOLM WATSON said that he hoped for greater things from a drug which would strike at the schizont rather than from one which struck at the gametocyte, and he was keenly interested, therefore, in "erion." He had drawn up a scheme of malaria-control for a large copper mine on the Congo Border well within the tropics, being only about 10° south. Anti-larval control had been so efficient that within two years malaria among Europeans for the month of November had fallen from 78 per 1,000 to 4·8 per 1,000. The number of Europeans was over 1,800. In addition there were over 10,000 Africans, among whom were some 1,500 children. We knew the spleen-rate and the parasite-rate of the different age-groups of the African children. This seemed to him a very unusual opportunity of experimenting with the new drug, because the experiments would be carried out under the enervating conditions of the tropics, which were entirely different from the conditions in England or in the temperate zones.