REPORT ON TWO EXPERIMENTS

ON

THE MOSQUITO-MALARIA THEORY

INSTITUTED BY

THE COLONIAL OFFICE AND THE LONDON SCHOOL OF TROPICAL MEDICINE

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THE MOSQUITO-MALARIA THEORY.

THAT there might be some connection between mosquitoes and the intermittent fevers peculiar to certain swampy regions was to all appearances a popular belief amongst the ancients, just as it is at the present day in Italy, in the southern Tyrol, and amongst certain tribes inhabiting intensely malarious areas within the tropics. It is, therefore, a mistake to attribute the mosquito-malaria theory to Lancisi, Nott, King, Laveran, or Bignami; these men merely supported the old belief with that power of conviction which they had derived from a wide and enlightened experience of malarial epidemiology.

The discovery made by Manson twenty years ago that a mosquito is the intermediary host of *Filaria Bancrofti*, Cobbold, and the more recent discovery by Smith and Kilborne that *Piroplasma bigeminum* is propagated by a common tick of cattle, the *Rhipicephalus annulatus*, added to the mosquito-malaria theory the support of analogy.

It is to the combined efforts of Manson, Ross, Grassi, Bignami, and Bastianelli that we owe the scientific demonstration of this theory.

Manson pointed to the fact that the flagellation of gametes occurred only in abstracted blood, as indicating the probability of an exogenous cycle in the life-history of the malaria parasites within the body of some blood-sucking alternative host, and suggested that the mosquito might probably be the definitive host of these parasites, because of its wide geographical distribution, of its habits, and of its limitation to swampy areas. He further suggested that possibly each kind of malaria parasite might require a special species of gnat as its alternative host.

Ross, following up Manson's suggestions, discovered that certain mosquitoes were indeed the definitive hosts of the malaria parasites, and that they contracted and transmitted the infection directly by means of their bite. In 1897, he discovered the early encysted form of the æstivo-autumnal parasite within the stomach wall of certain gnats of the genus *Anopheles* which he had previously fed on malaria patients. Being unable to continue his investigations with human malaria, he turned his attention to the *Hæmamœbidæ* of birds, and found in *Culex fatigans*, fed on sparrows or larks containing *Hæmamœba relicta*, zygotes precisely similar to those of *Hæmomenas præcox*, which he had already seen in anopheles.

It remained now to follow out the life-history of the zygotes, and this Ross did with the avian parasites. He saw that they increased in size, divided, and became full of filiform spores, then ruptured and poured out their multitudinous progeny into the body-cavity of their insect host. Finally, he saw the spores accumulate within the

cells of the salivary glands, and discovered that they actually passed down the salivary ducts into the seat of puncture, thus causing infection in a fresh vertebrate host.

Repeating Ross's researches and experiments with the parasites of human malaria and with mosquitoes of the genus Anopheles, Grassi, Bignami, and Bastianelli were soon able to prove that *Hæmamœba malariæ*, *Hæmamœba vivax*, and *Hæmomenas præcox* go through precisely the same transformations as *Hæmamœba relicta* within the body of their respective insect hosts.

The assertion made by Grassi that he had arrived at the demonstration of the mosquito-malaria theory quite independently of Ross's researches led to some misunderstanding as to the priority of the discovery; but, if some of the Italian papers attributed to Grassi this discovery as well as the invention of mosquito-netting, it can be positively stated that no such misunderstanding prevails amongst men like Marchiafava, Celli, Bignami, and Bastianelli, all of whom have given full credit to Ross's work.

The part taken by the Italians in the investigation and elucidation of the mosquito-malaria theory is certainly an important one, and the promptness and ability which they have shown in grappling with the consequent all-important question of prophylaxis is really admirable.

The first in the prophylactic field was Prof. Celli, who, in 1899, started a series of experiments to test the best methods of protection against the malaria-bearing mosquitoes.

Previous Experiments.

The experiments made by the Italian physicians were quite conclusive, and proved beyond doubt the correctness of the mosquito-malaria theory as regards the parasites of human malaria. However, as a means of carrying full conviction to the mind of the general public throughout

the world, they offered some disadvantages. The experiments made by Bignami, Bastianelli, and Grassi to prove that the malarial fevers are transmitted by the means of mosquito bites were carried out in the city of Rome, which, although now usually free from the disease, is nevertheless in the very heart of an intensely malarious region. And those made by Celli were invalidated by the fact that the people placed under protection had suffered from malaria in previous years, and that quinine was administered to them as a prophylactic. The object of Celli's experiments was not to prove that by avoiding the puncture of mosquitoes it was possible to escape fever, but to investigate all such means as might be destructive to mosquitoes, prevent their becoming infected, and protect from their bite.

Object of the Experiment.

For these reasons, and on account of the great importance of the question to the welfare and prosperity of most tropical colonies and protectorates, the Colonial Office decided to make an experiment, which, carried out on strictly scientific lines, might prove in the most convincing and striking manner the truth of the mosquito-malaria theory, and show how far the necessary protection might be consistent with the ordinary avocations of life.

The experiment was suggested by Dr. Manson, and supported by the London School of Tropical Medicine. It was to consist of two tests which should prove the theory, one in a direct positive manner, the other by negative inference. Should they both succeed, no argument could be reasonably brought against the correctness of the theory.

For the first test, a man who had never suffered from malaria, and who had never been abroad, should allow himself to be bitten by mosquitoes reared and infected abroad, and then sent to London.

For the second test, two men who had never suffered

from malaria should go to one of the regions best known as undoubtedly and severely malarious, and should live there throughout a fever season with no other protection than such as might prevent the bite of mosquitoes. For the purpose they would be provided with a mosquito-proof hut.

In a lecture delivered at the Colonial Institute on the 13th of March, 1900, Dr. Manson announced the approach of these experiments, and ventured to prophesy that the man bitten by the infected mosquitoes sent from abroad would contract the disease, whilst the two men living in a very hotbed of malaria, but protected against the bite of mosquitoes, would escape.

Manson's own son, Mr. P. Thurburn Manson, volunteered for the first test, and the second one was entrusted to ourselves.

It was decided to make the second experiment in the Roman Campagna, a district so well known as the classic land of malaria, not only on account of the ravages of the endemic, but also because of the noble work accomplished by the Romans during their long struggle with the disease. Moreover, in the Campagna, the experiment could be closely watched, as indeed it was. It had the further advantage of comparative proximity to London, a circumstance necessary for the first experiment.

In the Roman Campagna, the true fever season usually begins in July, and lasts till the autumn rains, which occur about the middle of October. It was arranged, therefore, that everything should be got ready for the experiment to commence by the end of June. The choice of a suitable locality, the many formalities necessary before the ground could be occupied, and the difficulties of getting a sufficient number of skilled workmen for the erection of the hut, made it impossible to begin the experiment before the middle of July. However, this delay was of little consequence, because the fever season was also delayed on account of unfavourable meteorological conditions.

The month of June was chiefly spent in visiting various parts of the Campagna in order to study the country, the habits of its people, and the conditions favourable or inimical to the malarial endemic. In the many excursions, various kinds of animals were collected, and their blood examined for such parasites as might tend to throw light on those of human malaria. At the same time, the rearing of mosquitoes for the inoculation experiment, and the arrangements most suitable for their transport to London, received all possible attention.

Conferring Malaria by Mosquito Bite.

The first batch of mosquitoes sent to London was reared from the egg in Prof. Bastianelli's laboratory in the Santo Spirito Hospital. The insects were fed on a patient suffering from benign tertian fever, and sent to London on the 29th of June. The case on which the mosquitoes were fed was selected by Prof. Bastianelli, who had found a few tertian gametes in its blood two days previously. On the actual day of feeding we examined several slides of the patient's blood, but failed to find any parasites. However, the mosquitoes were forwarded more to test the efficiency of the method of transport than with any great hope that they would confer malaria.

After that date, owing to difficulties in getting the required number of laboratory-reared mosquitoes when wanted, Bastianelli's assistant went to Porto and collected a large number of young Anopheles which had only just emerged from their pupal cases, and had not fed, to use for the experiment. Although assured that mosquitoes thus collected had already been successfully employed for inoculation experiments by the Italian physicians, we stopped the forwarding of the insects until we had thoroughly satisfied ourselves that we could easily and certainly distinguish the newly emerged and unfed imagines from those which had already fed on blood or plant juices. Having ascertained this to be the case, it

was decided to employ the insects thus collected whenever it was found impossible to obtain laboratory-reared specimens. All the Anopheles sent home after the first lot were collected in the imago stage on the margins of the Porto swamp, which had been at one time the famous hexagonal dock built by the Emperor Trajan. These Anopheles were all of the species *maculipennis*, and each single insect was carefully examined by ourselves. They were all infected on cases of benign tertian fever, this type of malaria being selected because it offers no real danger, and is easily cured by quinine. The patients employed for the experiment were very carefully and repeatedly examined by Prof. Bastianelli, Dr. Panichi, and ourselves, so as to absolutely exclude the possibility of confering any other type of malarial infection.

The second batch of Anopheles was sent to London on the 23rd of August. It numbered twelve insects, some of which had been fed twice or three times on the same patient, a boy suffering from a double benign tertian infection with numerous gametes in the blood. The third batch, numbering fifty specimens, was sent to London on the 7th of September. About thirty of these mosquitoes had been fed on a case of benign tertian. The others, which had refused to bite, were placed in a separate tube labelled "non-infected," and were also sent for control experiments, or to be infected in London if a suitable case offered.

Method of Packing and Transmission.

The transmission of the infected mosquitoes from Rome to London was a matter of some difficulty. The insects had to be kept a certain time until they had been fed on a suitable patient, then they had again to wait in readiness for the Indian mail from Brindisi, the use of which had been kindly permitted by the Post Office authorities. As soon as notice reached Rome of the arrival of the mail at Brindisi, a servant of the British

Embassy in Rome carried the insects along with the Ambassador's despatches to Ancona, where they were delivered to the officer in charge of the mail. An attendant of the London School of Tropical Medicine met the mail at Charing Cross and conveyed the insects to their destination. The journey from Rome to London lasted about three and a half days. So many unfavourable conditions of temperature, shaking, etc., having to be experienced on the journey, it was necessary to devise some apparatus that would obviate as much as possible these risks. It was at first proposed to send the Anopheles in glass tubes, with cotton netting over the top to allow of the transmission of air; but this did not seem a suitable means of conveyance on account of the shaking and jolting to which they would certainly be exposed. Having noticed in our many visits to the houses and stables in the neighbourhood of Ostia that the Anopheles there collected very often, and seemingly by preference, rested on the old cobwebs, and maintained their position on these delicate structures with ease even while the latter were wafted about by fairly strong currents of air, we thought it advisable to reproduce in our arrangements for transmission as far as possible a similar condition. Mosquito netting was loosely applied round a framework of tinned wire, so as to form a cylinder eight and a quarter inches long by three and a half inches in diameter, which might be closed at both ends by means of strings. Four such cylinders were placed within a square wooden box to protect them from damage, to render them more portable, and to keep the insects in a state of semi-obscurity. On each one of the sides of the box was made an opening three inches square, covered with wire netting to allow of the transmission of air, and also to prevent the mosquitoes escaping should the cotton netting in any way get torn. By means of this method of packing, with very few exceptions, most of the mosquitoes arrived in London alive and in good condition. All the mosquitoes that chose were allowed to feed again on the day of departure, so as

to keep them in as good a state of nourishment for the journey as possible. As a precautionary measure, especially for those that had not fed on the day of

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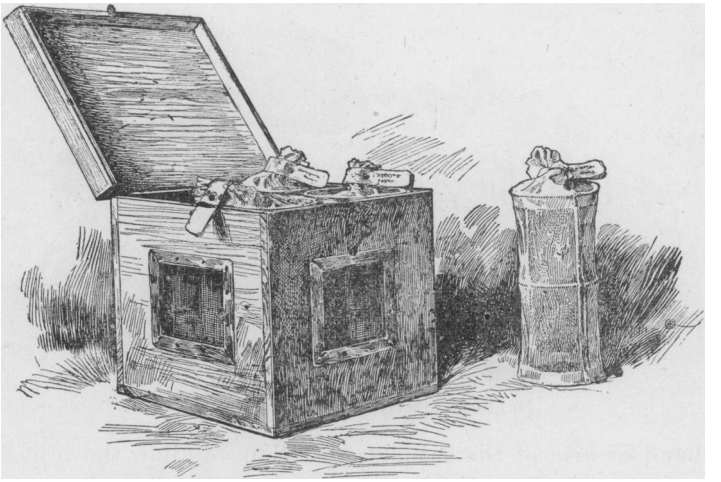


Dr. Sambon's travelling cage for mosquitoes.

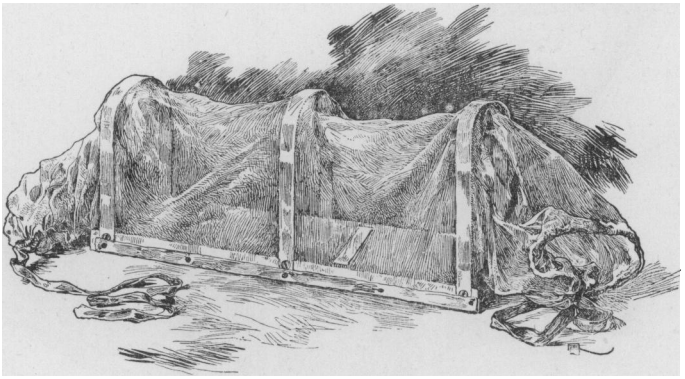
departure, slices of fruit (plum, apricot, or water-melon) were placed beneath each cylinder in such a way that the insects might, if they chose, suck up the juices through the meshes of the netting.

METHODS OF INFECTION.

The aforementioned gauze cylinders were devised simply for the transport of the mosquitoes, and were not large enough to allow of the introduction of the hand. Therefore Dr. Rees, Superintendent of the London School of Tropical Medicine, devised a special apparatus to allow the insects to puncture the man to be experimented upon. To a wooden board on which the hand and arm could be applied, a cradle of cotton netting supported by thin strips of metal was fixed, and the Anopheles liberated inside it. However, as some of the mosquitoes might escape or be killed on withdrawal of the hand, it was

found, on account of the comparatively small number of infected mosquitoes available, that the best method was to place the cylinders in which they had travelled on the

FIG. 2.



Dr. Rees' infecting cage for mosquitoes.

hand or arm of the subject, and thus to allow the insects to puncture through the meshes of the netting.

P. THURBURN MANSON'S CASE.

Mr. P. Thurburn Manson, who had volunteered to be bitten by the infected mosquitoes on their arrival in England, submitted to the puncture of the three different batches. The first batch turned out to be non-infected, as we had supposed from the absence of parasites in the blood of the patient on whom they had been fed; but the second consignment proved successful, and Mr. Manson passed through a sharp attack of double benign tertian fever. Careful notes on the experiment were taken by himself, and are here given *in extenso*.

NOTES OF EXPERIMENT *by* P. THURBURN MANSON, *Guy's Hospital*.

"I am twenty-three years of age, was born in China, but have lived in this country since I was three; have never

been abroad since, nor in any district in this country reputed to be malarial. I am healthy.

The first consignment of mosquitoes arrived at the London School of Tropical Medicine on July 5th. They were in a languid condition, and would not feed satisfactorily. One may have bitten me. By July 7th they were all dead.

The second consignment arrived on August 26th. On arrival twelve insects were lively and healthy-looking. I fed five of them on August 29th, three on August 31st, one on September 2nd, and one on September 4th. They bit my fingers and hands readily. The bites were followed by a considerable amount of irritation, which persisted for two days.

The third consignment arrived on September 10th. There were some fifty to sixty mosquitoes in good condition. Twenty-five bit me on September 10th, and ten on September 12th.

Up till September 13th I had been perfectly well. On the morning of the 13th I rose feeling languid and out of sorts, with a temperature of 99° F. By midday I was feeling chilly and inclined to yawn. At 4.30 p.m. I went to bed with severe headache, sensation of chilliness, lassitude, pains in the back and bones, and a temperature of 101·4°. Repeated examinations failed to discover any malaria parasites in my blood.

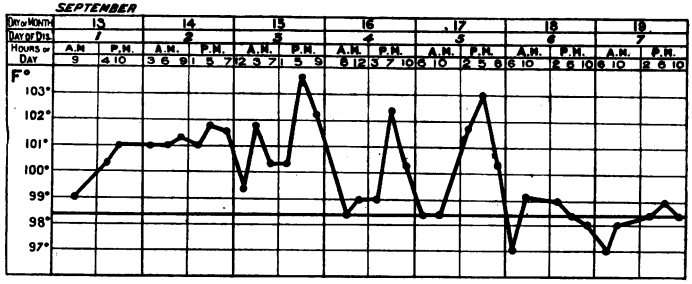
September 14th.—I slept fairly well, but woke at 3 a.m. with slight sweating and a temperature of 101°. During the day my temperature ranged between 101° and 102°. The symptoms of September 13th were exaggerated, and anorexia was complete. Several examinations of the blood were made again with negative result. To relieve headache 10 grs. of phenacetin were given at 6 p.m.; I perspired profusely but slept indifferently.

15th.—Woke at 7 a.m. feeling distinctly better, with a temperature of 100·4°. No malaria parasites were discovered on repeated examinations of my blood by my

father. About 2 p.m. I commenced to feel slightly chilly; this soon wore off, and I became hot and restless. By 4.30 p.m. temperature was 103·6°. It remained about 103° till 9 p.m., when profuse sweating set in. I am told there was some delirium.

16th.—I woke at 8 a.m. feeling quite well; temperature 98·4°. I made several blood examinations, and found one doubtful half-grown tertian parasite. In the afternoon and evening there was a recurrence of fever (temp. 102·8°), relieved by sweating.

17th.—Again felt quite well on waking after a good night's sleep; temp. 99°. At 10 a.m. several half-grown parasites, a gamete, and two pigmented leucocytes were discovered in the first blood film examined. During the



day many tertian parasites were found. Their presence was verified by my father, Dr. Frederick Taylor, Lieutenant-Colonel Oswald Baker, I.M.S., Dr. Galloway, Mr. Watson Cheyne, F.R.S., and Mr. James Cantlie, some of whom saw the films prepared.

About 2 p.m. the sensation of chilliness returned. Temp. 101·8°. By 5 p.m. temperature had reached 103°. There was then copious sweating. The edge of the spleen could be felt on deep inspiration, and there was a slight feeling of discomfort in the region of that organ. Dr. Frederick Taylor and Mr. Watson Cheyne confirmed the presence of splenic enlargement. By 9 p.m. the temperature had fallen to 99·2°, and I was feeling better. Quinine (10 grs.) was given.

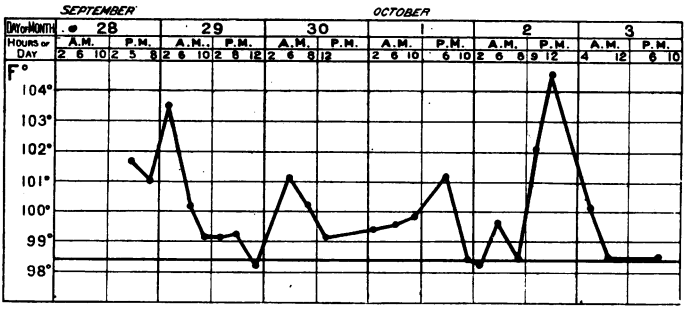
18th.—Woke after a good night feeling perfectly well (temp. 97°). Ten grains of quinine were taken, and subsequently five grains every eight hours. I continued perfectly well all day. A few three-quarter grown tertian parasites and some gametes were found during the forenoon and afternoon; they were seen by Dr. Oswald Browne, my father, and myself. At 10 p.m. the parasites had disappeared, the last being found at 5 p.m.

19th.—No parasites discovered. Temperature normal. Feeling quite well. There is no splenic enlargement and no tenderness. Appetite returned.

25th.—In good health. No recurrence of malarial symptoms."

Warren's Case.

At the same time, Mr. Warren, assistant in the laboratory of the London School of Tropical Medicine, subjected himself to the puncture of the third batch of mosquitoes, namely, those which arrived in London on



the 10th of September. He underwent likewise a severe attack of benign tertian fever. The notes of his case were collected by Dr. Rees, and are as follows:

"Mr. Warren first began to feel ill on Friday, September 28th. The incubation period in his case seems to have been about fourteen days. His temperature on Friday afternoon was 101·6° F., but a careful

blood examination failed to reveal any malarial parasites. The next morning, however, a leucocyte was discovered which contained the characteristic malarial pigment. On Sunday, September 30th, a specimen of his blood was examined, and showed four leucocytes, which contained degenerating advanced pigmented parasites. On Monday, October 1st, he was feeling better, and his temperature was down. That evening, however, the thermometer registered 102°, and at midnight 104·4°. There was no definite rigor, but a distinct feeling of chilliness. The blood examination the next morning revealed young benign tertian parasites; four to six were present in each slide of blood examined. On the following day quinine was administered. The blood specimens in this case were seen by Dr. Manson and a dozen or more of the students working in the laboratory of the school."

Nothing could be more convincing than the history of these two cases in proof of the *rôle* played by mosquitoes in the propagation of malarial fevers. Neither of the subjects of experiment had ever suffered from malaria before; both were residing in a non-malarial country, and both, in consequence of the bite of Anopheles fed on patients of benign tertian in Rome, after a definite incubation period passed through typical attacks of benign tertian fever, and the corresponding parasites were demonstrated in their blood.

Protection from Mosquito Bite.

Choice of Locality.

Whilst these experiments were being made in London to prove that malarial fevers are propagated by mosquitoes, the other and collateral experiment was being carried out in the Roman Campagna. Here four persons were exposed to all those conditions which hitherto had been popularly regarded as causing malarial fevers, and

had no protection except wire netting against mosquito bites.

It had been arranged by Dr. Manson, Prof. Celli, and one of us that the experiment should be carried out at Cervelletta, a low-lying place in the valley of the Aniene, between Rome and Tivoli. This locality, undoubtedly malarious, is now being cultivated by a colony of Lombards, who have already given to a part of the district the appearance peculiar to the rich agricultural districts of their native province. However, when we arrived in Rome, at the beginning of June, we soon became aware that it would have been unwise to erect the hut with which we had come provided, in the Cervelletta district. Firstly, because our experiment would have been looked upon as part of Prof. Celli's experiment, which was being carried out in that locality; and secondly, because Cervelletta was not considered by all to be sufficiently malarious for our special object. We therefore consulted Marchiafava, Celli, Bignami, Grassi, Bastianelli, Gualdi, Dionisi, and other Roman physicians who have made a special study of malaria, and they all agreed that we could not do better than choose either Ostia or Maccarese for the site of our experiment, both places being situated at the mouth of the Tiber, on the low and swampy alluvial soil of recent formation, and both well known as intensely malarious.

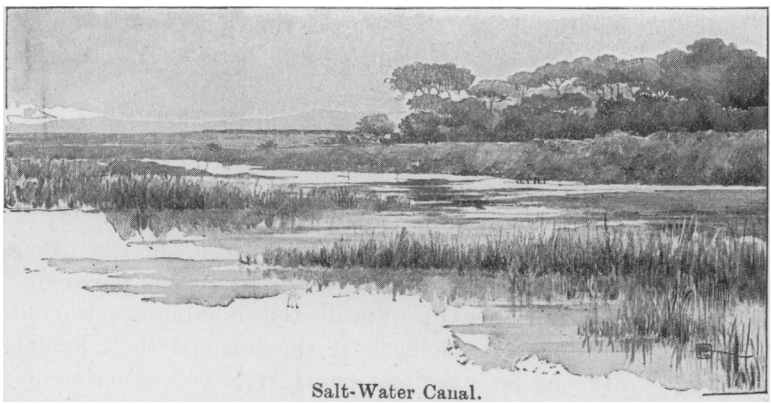
We accordingly visited these localities several times, and finally decided to erect our hut in the district of Ostia. A strong point in favour of Ostia was that it would be inhabited throughout the fever season by a number of Ravenna colonists; Maccarese, on the other hand, would be almost entirely deserted at this particular time. The presence of unprotected inhabitants was of great importance to us, because the state of their health would serve as an excellent contrast to our experiment; and, moreover, a number of fever patients in the neighbourhood meant a larger percentage of infected mosquitoes, and therefore greater chances of infection.

Then, again, Ostia was far better known than Maccarese, having been the port of ancient Rome.

In the district of Ostia we chose the locality called Fumaroli, which the inhabitants of Ostia unanimously stated to be the deadliest spot in the neighbourhood. It is about a quarter of a mile to the south of the Ostia pumping station, which since 1890 drains the bed of the old swamp, and was itself the seat of an older pumping station built by Sig. Fumaroli.

The hut was erected on a narrow stretch of ground situated between the new road leading to Castel Fusano and the outlet of the swamp called "Canale dell' acqua salata" (Salt-Water Canal), and bounded on the east by the half-drained swamp which is now thickly overgrown with canes, and on the west by a drainage canal, which discharges the low level waters from the reservoir of the pumping station into the Salt-Water Canal. The area thus circumscribed is at about sea level, and quite six feet below the road, which runs on a kind of embankment. It is entirely formed of sand. The Salt-Water

FIG. 3.



Salt-Water Canal.

Canal, in this part of its course, was at the time of the experiment almost choked by aquatic vegetation, and

behind it rose the pine forest of Castel Fusano, making a most effective background, but adding greatly to the unhealthiness of the place by affording shelter to the adult mosquitoes.

This locality belonged to the State, but was rented by the King, so that we had to obtain permission from His Majesty to erect our hut there. The late King Humbert, with the greatest kindness, at once consented, and showed much interest in the experiment, promising to visit the hut, giving us permission to wander about his hunting preserves, placing his gamekeepers at our disposal, and ordering that every day the state of our health should be reported to him.

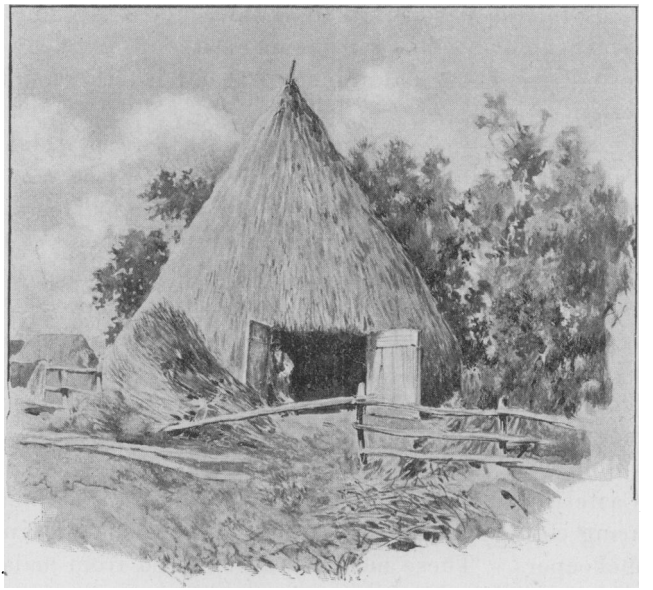
That this locality well deserved its bad reputation was fully evidenced by the sickly appearance of the inhabitants, and by the enormous number of Anopheles larvæ in the surrounding drainage canals, in the pools within the pine forest, and especially in that part of the swamp which had been only partially drained in order to serve as a cover for the wild boar and other animals haunting the preserves.

There were only four buildings in the neighbourhood, namely, Castel Fusano, the pumping station, Casa Fumaroli, and Casa Massei.

Castel Fusano itself was uninhabited, but in a neighbouring cottage lived a policeman and two of the King's gamekeepers. These men had all suffered from malarial fevers in previous years. About twelve years ago the bedroom windows of this building had been screened with wire netting by order of Queen Margherita as a protection against mosquitoes and breeze-flies, but only one window still preserved its netting in good condition. The netting had been wrenched from all the other windows long ago to permit the airing of clothes, the growing of sweet herbs, the desiccating of tomatoes, and the many other purposes to which windows are put in Italian households. All the beds were provided with roughly improvised, but fully efficient mosquito curtains.

However, notwithstanding this protection, and the free use of quinine for the slightest ailment, all the inmates of the cottage suffered again this season from slight attacks of fever. The fact is that their means of protection were only nominal; the men used to sit out in the garden during the evening, and were thus frequently bitten by mosquitoes.

FIG. 4.

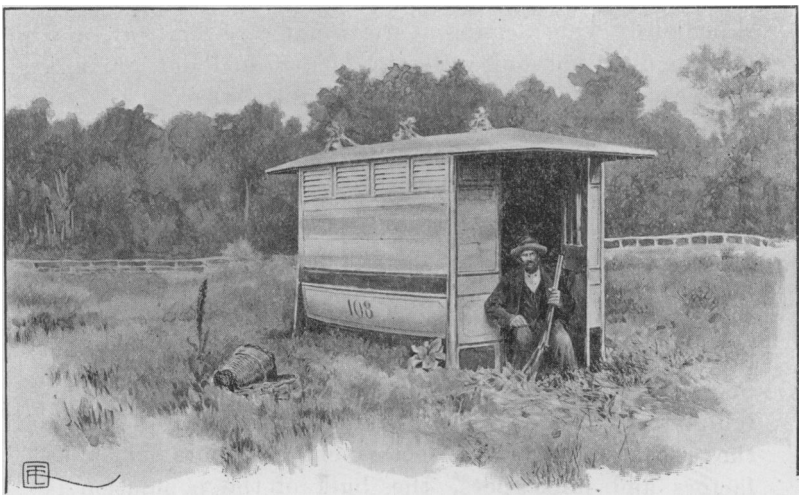


Reed hut near Ostia. (Drawn by A. Terzi.)

The pumping station is a fine substantial building, with lofty, clean, thoroughly ventilated rooms. It was inhabited during the summer by the superintendent in charge, who went very frequently to Rome, whither he had sent his family and servants. Except when on duty, the workmen slept in the village of Ostia. The superintendent, Sig. Celligari, was a most intelligent and amiable person, who took great interest in our experiment, and

helped us considerably in many ways. He remained to all appearance immune; he had suffered from well-marked malaria in previous years. During the current summer, although he felt ailing and feverish at times, no parasites were ever found in his blood. He made use of a mosquito curtain at night, and took quinine whenever out of sorts. Sig. Celligari was so much struck by the efficiency of our means of protection, that he asked his superiors to provide wire netting protection for the living

FIG. 5.



Old omnibus used as a dwelling near Castel Fusano. (Drawn by A. Terzi.)

rooms at the pumping station; and this being granted, he said that next year he should keep his wife and children in Ostia throughout the fever season. The workmen sooner or later all contracted fever, although they had been several years in the district and had suffered severely in previous years.

Casa Fumaroli was the building nearest to our hut, from which it was distant barely three hundred yards. It was inhabited by seven people, all of whom suffered

severely from malarial fever. They employed no means of protection and distrusted the use of quinine.

The furthest building to the east was Casa Massei, one of the small farmhouses built by the Ravenna colonists. This house provided us with quite a number of patients, because several successive batches of labourers inhabited it at different times, and all contracted fever.

Besides these buildings, there were a few huts in the neighbourhood, and some old omnibuses deprived of wheels and turned into dwellings. The inhabitants of these singular homes suffered severely with the exception of one man, the road-keeper Lupino, who had placed a ridiculously wide netting at the window of his hut, and had made a mosquito-curtain for his bed out of rags of every colour and description sewn together.

With the exception of a few scattered huts and farmhouses, there are no other habitations in the district save the village of Ostia. The latter consists of a few mean buildings clustered round an old mediæval castle, and of three large reed huts, which serve as stables for horses in summer, and as habitations for men in winter.

The population of Ostia amounts to several hundred inhabitants in winter, but dwindles down to about fifty in the fever season. Properly speaking there is no indigenous population in the district of Ostia. With the exception of a few permanent residents, such as factors and innkeepers, the bulk of the population is formed by peasants from various parts of Italy, who come at different times for the various field works. The greater number arrive about the middle of October for the ploughing, the sowing, and the weeding of the land, and leave again in June just before the malarial season begins. These peasants come chiefly from the Abruzzi and from Sora. They congregate in large reed huts very elaborately partitioned, which are thoroughly smoked every evening, and sometimes during the day, by wood fires lighted inside for cooking and washing purposes. Thus, by avoiding the fever season, and by keep-

ing mosquitoes out of their huts, most of these peasants escape malaria unless special meteorological conditions have unduly hastened or prolonged the epidemic.

Two other batches of workers from the Marche and from Umbria come, one in June for the wheat harvest, the other in September for the maize harvest. They sleep in small huts extemporised with boughs and straw, or under a piece of canvas stretched on poles, or in the open without any shelter whatever. These peasants, and especially those who come in September, suffer very severely from malaria.

Of these labourers many have returned to the district for several years in succession, or have worked in other malarious regions, and have therefore suffered previously from fever. But every year there are numbers of new-comers, especially amongst the younger folk, who have never had the disease. These invariably contract malaria after a very short time, which is often not longer than the necessary incubation period.

Of the old cachectics, a few, during some seasons, escape acute manifestations of the disease, as if they had acquired a kind of immunity against the infection. The greater number suffer from attacks of fever season after season, throughout a number of years, as if such immunity did not exist.

Beyond the statement of these facts we have nothing more to say about immunity, because the peculiar conditions of the population of Ostia did not allow of any satisfactory study on the subject.

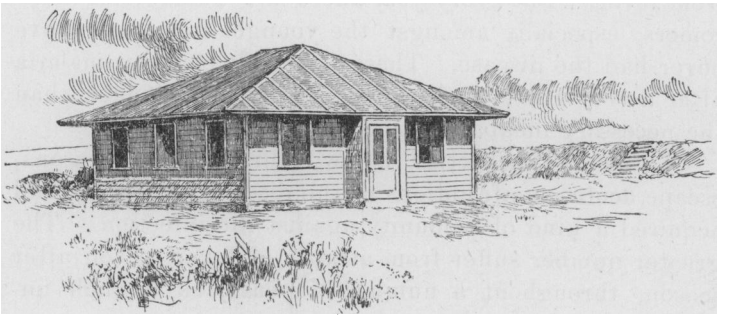
It has been frequently stated that it is sufficient to sleep one single night in Ostia during the fever season to contract the disease. There was no evidence for such a statement, beyond the somewhat vague and unreliable assertions of sportsmen and peasants. This year, however, soon after the assassination of King Humbert, about fifteen police-agents were sent to Ostia to arrest certain anarchists, who were believed to have taken refuge in that village. On the authority of Prof. Celli, who was

informed of the fact by the police medical officer, we may state that all these police-agents suffered from fever within a fortnight, although they had remained but part of a night in Ostia.

The Hut.

The hut for the experiment was supplied by Humphreys Ltd., London. The building throughout was constructed of yellow fir framing. The whole was erected at Humphreys Ltd. Works, all joints marked, taken down, packed, and delivered at docks for shipment to Rome.

Fig. 6.



British experimental hut.

The building was 32 feet long by 24 feet wide and 3 feet from floor to under side of ceiling, and had an entrance porch 4 feet by 3 feet. The plan consisted of a corridor 4 feet wide through the building, with three rooms on one side, and two large rooms and two small on the other. The foundation provided consisted of timber piles driven into the ground, the top being halved to receive the sleeper plates (six rows being supplied), which in turn carried the floor joists. Upon these the framing of the building was erected. The walls were constructed of 4-inch timber covered on the outside with special rebated weather-boarding with felt lining under, and internally with $\frac{5}{8}$-inch tongued, grooved, and beaded

matchboarding, having an air-space of 4 inches between outer and inner linings. The roof was designed and constructed with overhanging eaves of 3 feet all round, this being required to protect the building from the direct rays of the sun and also to assist the system of ventilation, the 3 feet overhanging eaves being left entirely open, and then covered with mosquito-proof wire. The roof was covered on the outside with 1-inch boarding and tarred felt upon it, the seams being secured with small wood fillets planted on, and when finally fixed covered with a cement composition. All rooms were flat ceiled at 8 feet, giving the whole space of the roof for ventilation, each room having a ventilator 2 feet 6 inches square in the ceiling, covered with wire; thus giving a thorough through current. The floor consisted of 1-inch tongued and grooved flooring, and was finished with a square edged skirting. The windows to all rooms were casements glazed with clear glass, opening inwards. The outside of windows over the whole of the opening was covered with mosquito-proof netting. The entrance doors to porch consisted of one pair 2-inch moulded and panelled doors, the upper panels glazed; and two feet further in the corridor was a partition across same with a single door leading through. All the doors to the rooms consisted of 1½-inch square framed and panelled doors on 1-inch linings, stops, and architraves, all complete.

All doors and windows were painted externally in oil-colour paint. Cast-iron eaves gutter with necessary fascia board was fixed to eaves all round the building, with outlets and cast-iron down pipe complete.

The interior of the building generally was finished in white paint, this being adopted for the easier detection of the mosquito. As an extra precaution, mosquito curtains were provided for each bed; they were placed at a certain distance from the bed, after the manner of a partition,—that is to say, two walls of cotton netting descended at right angles from ceiling to floor, thus

shutting in the corner of the room in which the bed was placed. One side, made of one large piece, was fixed all round by means of strips of wood nailed to the ceiling, the wall and the floor of the room; the other, which served as an entrance, was formed of two pieces hanging in such a way as to widely overlap each other. They were fixed on two sides, and at the bottom were kept closely applied to the floor by means of a weighting shot. The distance of the curtains from the bed prevented the possibility of mosquitoes biting through the meshes of the netting any limb which might be extended out of the bedclothes during sleep.

The cost of the hut, including transport from London to Ostia and erection, amounted to about £300.

At the end of the experiment the hut was presented to the Roman Municipality, which had borne the expense of erection, and had afforded us many other facilities.

In order to secure good drawings of mosquitoes, malarial parasites, and other objects of research, we engaged Sig. Terzi, a distinguished Sicilian artist, who agreed to live with us in the Campagna under the same conditions as ourselves throughout the time of the experiment. As a servant, we employed a man, Silvestri, who, though a native of Rome, had never suffered from malarial fevers. He had been a working naturalist, and proved very useful in collecting material for our work. Thus four people lived in the hut throughout the fever season with no further protection than that afforded by wire netting, and all remained perfectly well throughout the whole time.

This experiment was not only intended to corroborate a fact already scientifically demonstrated, but also to overthrow a number of fallacies and prejudices which prevented the full appreciation of the new discoveries in malaria, and impeded the advantages which would undoubtedly accrue from their practical application. The routine of our life in the Campagna was therefore greatly influenced by the object the experiment had in view. We had to prove that malaria was not carried by a

vitiated condition of the air, as the name of the disease implies, and therefore we always slept with our windows wide open, although the stench of the decaying vegetation and putrefying animal matter from the drying-up canals was very unpleasant at times. The people of the neighbourhood were greatly amazed at what they considered our temerity in exposing ourselves freely to the night air, and many in Ostia who heard of our mode of life would not believe it until they had seen it with their own eyes. To assure themselves on this point several came and walked round our hut in the early hours of the morning. The habit of the Romans of sleeping with hermetically closed windows probably originated on account of malaria, and is, of course, a perfectly consistent one, especially if viewed in the light of our present knowledge of the ætiology of this disease.

The water of malarious localities has also been considered an important vehicle of the disease, and many even now hold that it is so, notwithstanding the negative result of numerous experiments made by Celli, Brancaleone, Zeri and Marino, and the discovery of the *rôle* played by mosquitoes in the transmission of malarial infection.

The only drinking water in our neighbourhood was that of a draw-well in our own compound, and that of a pump-well near the pumping-station. Both supplies were undoubtedly polluted. During the erection of the hut one of us and the servant drank of the muddy well-water nearest our hut, and both subsequently suffered from diarrhœa, which was probably caused by this water. After that, all water from the locality was thoroughly boiled and filtered. However, boiled water was not used long for drinking purposes, because Signor Bisleri, of Milan, kindly presented us with an unlimited supply of his excellent table-water called "Nocera Umbra." The servant, throughout the whole period of our stay in the Campagna, continued to drink very recklessly any kind

of water, even that of ditches and pools, during our long thirsty excursions under the scorching sun of August.

Our food came chiefly from Rome, with the exception of bread, eggs, and sometimes milk and fowls, which were obtained in the neighbourhood. The people of the Campagna attach great importance to the food question in regard to malaria, and therefore it was not to be regretted that the difficulties of our commissariat obliged us to partake of a fare very similar to that of our malaria-stricken neighbours; in fact for a considerable period we messed at the pumping-station with the superintendent in charge.

We never took quinine, arsenic, or any other kind of remedy which might be considered prophylactic as regards malaria.

The turning up of the soil is another condition which has been looked upon as a source of malarial infection, on the erroneous supposition that the germ is capable of saprophytic life. The soil all round our hut was constantly being turned over for a number of reasons, amongst which was that of a possible archæological find. We were encouraged in this hope by the fact that while digging for the foundations of the hut we had come upon a tomb of the time of the Roman empire. It was formed of large terra-cotta slabs, and contained the skeleton of a young woman. Close to the base of the skull was a coin of the Emperor Commodus, evidently the toll-money for the passage of the Styx, which the old Romans used to place in the mouths of their dead. Our subsequent excavations only brought up a rude unfinished marble capital and a few terra-cotta fragments.

Our neighbours were certainly surprised at our immunity and good health, and they watched our experiment with keen interest; but when we advised them to adopt our simple and easy method of protection they shook their heads and invariably answered, "Wait till the rains come." The poor creatures spoke from cruel

experience, because a drenching is always followed by relapse in those who have latent malarial organisms.

When the rains came we went out to see what happened in the Anopheles breeding-grounds, and more than once we were thoroughly soaked by the torrential showers which overtook us in the open country, and the peasants saw us returning home cold and dripping; but no fever ensued, because we had no malarial parasites in our system that a chill might rouse into activity.

Probably for the same reason the inhabitants of Ostia warned us against sea bathing, and certainly the few sunburnt fishermen who spend the livelong day in shallow water collecting shellfish, and who live in miserable reed huts on the beach, suffer terribly from intermittent fevers. The cause of their illness was not difficult to find; all along the shore run parallel chains of sand dunes, amongst which are numerous Anopheles pools.

At St. Michael's Tower, near the mouth of the Tiber, were half a dozen grass huts raised on poles, like the pile dwellings of certain tribes of African and Oceanian savages. They were the habitations of a community of wretched reed-gatherers, who looked pictures of malarial wreckage.

At first we bathed very frequently in the waters of the Mediterranean; but in August and September it was almost worth a man's life to attempt to reach the surf through the pine forest or the jungle, on account of the hosts of breeze-flies which literally swarmed around us. The horses especially suffered terribly from these insects. To protect themselves somewhat from the sting of these flies the horses used to form into circles, with their heads towards the centre. The sight of these rings became very familiar. Sometimes forty or fifty horses were thus clustered in the middle of a field, and there they would stand throughout the hottest part of the day tossing their manes and lashing their long tails all round the circle.

Long experience in the Roman Campagna and in other malarial regions of Southern Europe has taught that the

most dangerous time for contracting malaria is during the evening twilight and at dawn. This fact agrees perfectly with the feeding habits of the mosquitoes of the genus Anopheles peculiar to these regions. We proposed therefore to retire an hour before sunset, and not again to leave our mosquito-proof hut until an hour after sunrise. But we very soon found that it was not necessary to retire so soon as an hour before sunset, because the Anopheles

FIG. 7.

Dwellings of rush-gatherers at the mouth of the Tiber. (Drawn by A. Terzi.)

appeared very punctually a few minutes after sunset and disappeared again a few minutes after sunrise.

During the day we went freely over all the ground within the vicinity of our hut collecting animals and visiting patients. Many people at home and abroad did not hesitate to express their opinion that we should contract fever by exposure in the daytime. Such a risk we soon found was infinitesimal so long as one knew the

danger and guarded against it. At first we were, of course, very careful, because in the interest of the experiment we were obliged to take every possible precaution against mosquito bite, and we made veils of cotton netting to protect our heads, and we wore thick woollen gloves to cover our hands, but very soon we went through woods and jungles unprotected without the slightest fear.

On the 7th of August a military balloon having stranded in the very centre of the swamp, after vain attempts to descend in a neighbouring field so as to avoid being blown on to the pine forest or the sea beyond, we felt obliged to run to the rescue of the aëronauts. Accordingly we entered the thick cane jungle which occupies the half-drained bed of the swamp. For more than two hours we struggled through the canes, which towered several feet above our heads. The heat was unbearable, and when we came out of the thicket with the tattered balloon we were quite exhausted, nevertheless to our surprise we had not been bitten. Had it not been our duty to give assistance, we should never have dared enter that jungle. In fact, up to that date we had carefully avoided coverts.

However, we never sat under cover, especially near pools and ditches, unless we wanted particularly to capture mosquitoes. As illustrative of the improbability of being bitten during the day in the Roman Campagna, we may say that in our many attempts at obtaining specimens of Anopheles peculiar to forests and jungles, we were only able to capture very few specimens.

Although, as a rule, we retired before sunset, still on three occasions one of us was obliged to go to Rome to despatch mosquitoes to London, and on other business, and consequently had to return late in the evening. Each time on the return journey passing through the village was avoided, and a cotton net round the head and woollen gloves were worn. Thus safety was insured, although the drive was for over a mile along

the canal through a veritable cloud of mosquitoes and other insects.

The experiment lasted from the 19th of July to the 19th of October,—that is to say, during the whole of what is considered the season of infection. In fact, the first new cases do not begin before the end of June, and fresh cases after the end of October are exceptional. The length of the season varies somewhat in different years accordingly to the meteorological conditions. Its duration is specially influenced by the earlier or later onset of the autumn rains.

Before our departure already hundreds of people had returned to Ostia; and three days before we left the heavy rains set in.

During the whole time of the experiment, and up to the present day (January 15th, 1901), none of us has had the slightest touch of malarial fever. Dr. Rees, who lived with us for a fortnight in September, and Sig. Gualdi, a medical student, who frequently stayed a night or two with us and kindly helped us in collecting material for our studies, also remained quite well. Our health was perfect during the whole time with the exception of the attack of diarrhœa already referred to, from which one of us and the servant suffered for a few days. As the result of our experiment our health may be said to have improved, notwithstanding the long fatiguing excursions in the Campagna, the great heat and moisture of our encampment, and the poor quality of the food we were able to procure.

During our stay in Ostia we were visited by a number of people; amongst these were the English physicians residing in Rome—Dr. J. Brock, Dr. Burton-Brown, Dr. Eyre, some of the most eminent Italian physicians, such as Prof. Celli, Prof. Grassi, Prof. Bastianelli, Prof. Rossi, Prof. Postempsky, Dr. Gualdi, Dr. Noè, Dr. Foa, and two distinguished German physicians, Dr. Plehn and Dr. Supitza, who had come to Rome for special malarial investigations.

Professor Grassi was so much impressed with the strictly scientific manner in which our experiment was being conducted that he sent the following telegram to Dr. Manson:

"Assembled in British experimental hut, having witnessed perfect health experimenters amidst malaria-stricken inhabitants. Italian physicians congratulate Manson, who first formulated mosquito-malarial theory." —GRASSI.

PHYSICAL CONDITION OF NEIGHBOURS.

The habitation nearest our hut was, as already stated, Casa Fumaroli, at a distance of about 300 yards. In this place lived the Monaldi family, consisting of two men, two women, and three children. All of these suffered repeatedly from malarial fever during the summer, notwithstanding the quinine treatment of each attack. It seemed as if these people were being infected anew again and again. With the exception of one of them, malignant parasites were demonstrated in their blood. They had all suffered in previous years, and showed very evident signs of malarial cachexia. Signora Monaldi had an enormous splenic tumour. The condition of one of the children became so grave that he had to be transferred to the St. Spirito Hospital, in Rome. Casa Fumaroli was always full of Anopheles from the adjacent canals. In the kitchen a number of fowls used to roost at night in a partially closed recess under a stair leading to the bedroom above. This place invariably teemed with fully gorged Anopheles. The women told us that the younger fowls seemed to suffer considerably from bites of these insects, but the blood of such of the fowls as we examined showed no parasites.

Most of the people in our neighbourhood were cachectic, and the peculiar facies of the disease was more or less apparent in all the residents. It was really a pitiful sight to go through the deserted village on a glorious Septem-

ber day and only meet with a few wretched, sallow-faced people closely wrapped in their coarse woollen mantles, who with a tired, absent look tendered us their dry, feverish hands to feel. The contrast between these peasants and ourselves was startling; or between them and the doctors of the malarial ambulances of the Red Cross and their attendants, all healthy and buoyant. The cause of the striking difference in our respective conditions was simply the few yards of wire or cotton netting we used to protect ourselves from mosquitoes. What proof of the efficacy of such means could be better than that supplied by Lupino, the road keeper, who made himself a mosquito curtain of patched-up rags and remained quite well throughout the fever season?

All the unprotected newcomers who remained in Ostia during the summer suffered from severe attacks of fever in the early period of the endemic. Of these, two men who came as factors to the Ravenna Colony were of special interest on account of the good condition of their health at the outset, and the comparatively hygienic circumstances under which they lived. One of these, Signor Francia, was very reckless; but the other, Signor Bonivant, was rather anxious about his health, and took quinine as a prophylactic. Early in July both were attacked with quotidian malignant fever, and were obliged to leave the district for some time.

Several patients came to our hut for quinine or advice, and the Red Cross doctors, especially Dr. Bartera, brought us blood-films from the patients they had found amongst the harvesters and conveyed to the hospital. Thus we were able to examine the blood of about fifty patients. In most cases parasites were easily found, but in some cases parasites could not be demonstrated, doubtless on account of the large doses of quinine which the patients had received from the municipality doctor, the Red Cross doctor, or their employers. In the early part of the season, tertian parasites were usually found, but later the malignant parasites were by far the most prevalent. In

September, a case of quartan fever was detected in a labourer working in the maize fields of Calabresi's estate, but this fever had been contracted out of the district. No other cases of quartan fever came under our notice or were described to us by the doctors or the peasants themselves. Several cases of mixed infection of tertian and æstivo-autumnal parasites were observed. In cases of æstivo-autumnal infection crescent bodies were never found; this may be explained by the fact that the patients who came under our observation were in the early stages of the infection,—that is, at the time when the gametes are not yet present. The crescent bodies seem to appear only after several febrile attacks, when probably the endogenous multiplication of the parasites is opposed through acquired resistance.

Numerous Anopheles were collected from houses, huts, and stables. Many of these were dissected, others were sectioned; very few were found to be infected with malaria parasites. Of fifteen collected one day from Casa Fumaroli, at a time when the inmates were suffering most from malaria, only two were found to have parasites, and these were in the early stages of development. However, we do not think that our observations on this point were sufficiently numerous to allow of any definite statement as to the relative number of infected insects. The Anopheles do not seem to remain long in rooms in which they are likely to be disturbed. Their number in the same varied very greatly from day to day.

The Mosquitoes of the District.

Our studies as regards the mosquitoes of the district were principally limited to those of the genus Anopheles. Of these, only two species were found, namely, *A. maculipennis* and *A. pseudopictus*. Although *A. bifurcatus* is said to be found in the locality, we did not come across a single specimen.

A. maculipennis (syn. *A. claviger*, *A. quadrimaculatus*

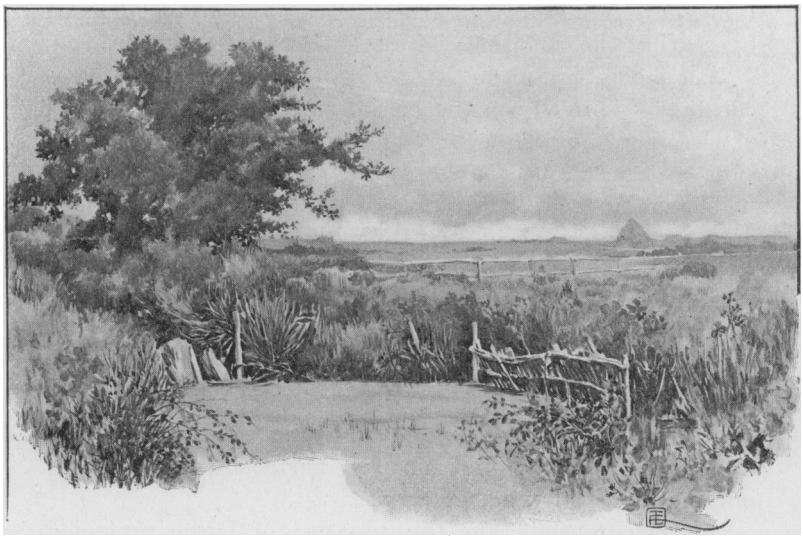
Pl. XXI, figs. 1—3, and Pl. XXII, fig. 3) is by far the commonest Anopheles of the region. Like *Musca domestica* and other insects, it has linked itself with man, and is now found in all the houses and stables placed in the neighbourhood of its breeding grounds. It is therefore, without doubt, the chief propagator of the malarial fevers of the region. Its larvæ were found in almost every water collection, from the broad outlet of the swamp to a small tub half full of water near the pumping station. The region is being partially reclaimed, and is intersected by a vast system of drainage canals, which during the summer were stagnant and overgrown with aquatic vegetation. Larvæ of Anopheles were found in the majority of these canals, especially on the leeward side of bridges.

At first we thought that the larvæ found in the Salt-Water Canal had probably drifted into it with the current, because they were chiefly found amongst a veritable *sargasso* of floating weeds close to the leeward bank. But a more careful observation showed that this was not the case. In fact, there was no current of water in the canal during the summer, because the mouth of it was entirely blocked up by a wide extent of sand, which had been thrown back by the sea. Moreover, the little shallow bays and pools made by the union of clumps of reeds along the banks of the drying-up canal were all plentifully stocked with newly hatched larvæ. In the brackish water near the closed-up mouth of the outlet, amongst large brown clumps of floating algæ and seaweed, Anopheles larvæ were very plentiful. Here, most of them were of a dark colour, some being jet-black, with milky white spots on one or more segments along the back.

In pools entirely covered by duckweed (*Lemna*) Anopheles larvæ were never found (Fig. 8).

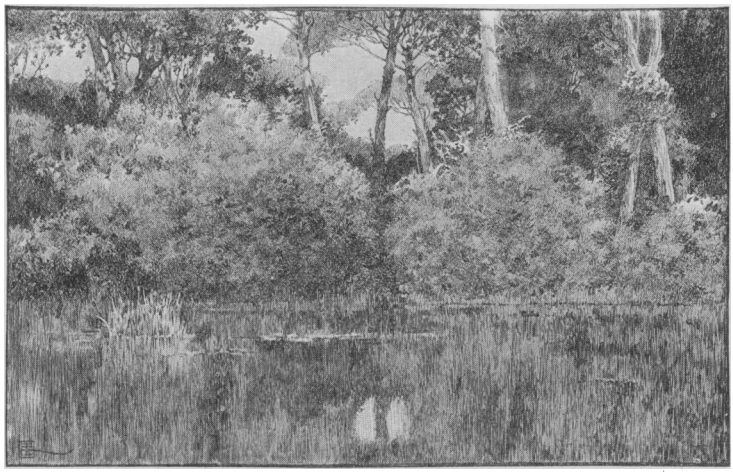
In a pool within the pine forest of Castel Fusano, and in some pools and ditches within the cane jungle beside it, we also found larvæ of *A. pseudopictus*. The distinction between the larvæ of *A. maculipennis* and those of *A.*

FIG. 8.



Pool entirely covered by duckweed, and consequently free from Anopheles larvæ. (Drawn by A. Terzi.)

FIG. 9.



Anopheles pool in pine forest. (Drawn by A. Terzi.)

pseudopictus was quite easily made out with a low magnifying power by applying Grassi's test, and was confirmed by rearing the imagines of both species from the larva.

Anopheles larvæ in all stages of development were constantly found from the beginning of June to the end of October, thus showing a continuous irregular succession of generations.

The number of larvæ in the different pools and at different times varied greatly without apparent reason. A striking instance of this was that of a small pool in Castel Fusano, close to the wooden hut in which King Humbert used to have his luncheon when out shooting. On the 20th of September, this pool contained absolutely no larvæ either of Culex or of Anopheles, but a fortnight later it was found to be simply teeming with both in all stages of growth, and on its surface were floating innumerable Culex egg-masses.

After torrential rain the number of larvæ remained almost the same in those pools which were under cover of trees and were not liable to be scoured out by the rain, on account of the depth of their banks, but they diminished somewhat in the smaller shallow pools in more open places, and almost entirely disappeared from the outlet after its mouth had been opened and a strong current established by means of the accumulation of water due to the rain and to the pumping operations.

In all the pools and canals were enormous numbers of frogs, besides swarms of larvæ of dragon-flies and water-beetles. The outlet and the wider canals contained, moreover, several species of fish, amongst which the young of grey mullet (*Mugil cephalus*) were innumerable, but the Anopheles larvæ occupied the shallows at the edge of the stream, where they were greatly protected by the dense aquatic vegetation.

Wishing to test the relative value of natural enemies as regards the destruction of mosquito larvæ, we made a few experiments with the larvæ of various water-beetles and other insects, and we found that the larva of a very

common and beautiful species of dragon-fly (*Crouthionis erythrea*, Pl. XXII, fig. 1) was especially deadly to mosquitoes. The odonata larvæ when provided with Anopheles larvæ in small glass vessels certainly devoured a considerable number of the latter. Such experiments, however, are of little value, as they do not reproduce the natural conditions. In fact, it cannot be said that the larvæ of this dragon-fly would not have taken other food by preference in their natural breeding grounds, as is most reasonably suggested by the size of their curious prehensive organ usually called a mask. The fact was certainly evident that enormous numbers of mosquitoes reached their adult stage, notwithstanding the extreme abundance of their natural enemies.

No experiments were made with larvicides, but we are convinced that the vast extent of ground covered by innumerable pools, swamps, and canals, usually thickly overgrown with aquatic vegetation, offered such difficulties that any attempt of the kind would have been hopelessly futile. Undoubtedly there are collections of water suitable to the kerosene treatment, especially in the neighbourhood of habitations, but many of these could preferably be filled up, drained, or stocked with small fish.

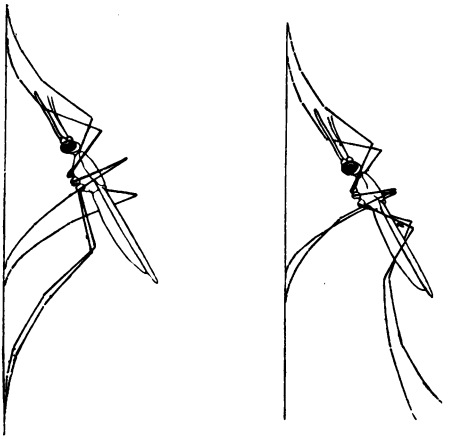
A most important matter in the abolition of mosquitoes is the clearing of canals and streams from water weeds, which offer the best breeding conditions. In the Roman Campagna and in the Pontine Marshes the wider canals are cleared by means of herds of buffaloes, which are driven into the stream and followed by men in punts, while other men with dogs run along the banks to prevent the buffaloes from landing.

As already mentioned, *A. maculipennis* was found in great numbers in the houses, chicken coops, and stables, in the last places often resting on the old dusty cobwebs which heavily curtained the ceilings. In the houses they chose the darkest corners, often resting under tables, beds, and chairs, but more frequently on the ceilings,

especially when these were blackened with the smoke of winter fires and well out of the way of danger. In the rooms of an inn which had a blue stripe all round their whitewashed ceilings, the Anopheles seemed to settle by choice on the dark stripe.

A. maculipennis usually, when resting on a vertical surface, sits at an angle of about 30°, the angle, however, varying slightly according to the position of the legs, the development of the ovaries, and the state of engorgement. Although this angle is much wider than that assumed by the local species of Culex, it is certainly not sufficiently marked to enable anyone to recognise the genus at a

FIG. 10.



Resting position of *Anopheles maculipennis*.

glance. A really characteristic difference between the Culex and Anopheles genera is that pointed out by Mr. Waterhouse. In Culex the head and thorax form an angle with the abdomen, which gives the insect a curious hump-backed appearance; in Anopheles the head, thorax, and abdomen are almost in a straight line.

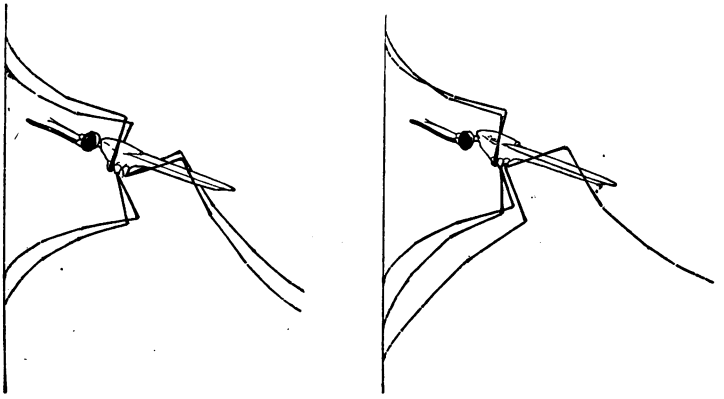
Having reared adult specimens of *A. pseudopictus*, we observed that they sit on a wall at a much wider angle than *A. maculipennis*. In fact, the body and the support

usually form an angle of about 70°, at times attaining almost 80°.

As some importance has been given to the resting attitude as a means of distinguishing the genera Culex and Anopheles, it is well to state that this distinction has no value in the diagnosis of genera, although it may be very useful in distinguishing species.

In the month of July adult specimens of *A. maculipennis* were very abundant, and continued so till the middle of September, when four days of torrential rain greatly diminished their numbers. After this, however, the number again increased, and remained fairly constant till the middle of October, when the weather became cooler and the autumn rains set in. In July and August no Anopheles were seen round the hut during the daytime, but in September and October, when there were several dull and cloudy days, a few stragglers occasionally appeared about the windows. On the whole, however,

FIG. 11.



Resting position of *Anopheles pseudopictus*.

their times were very constant, the time of appearance being a few minutes after sunset, and that of disappearance soon after sunrise. During the day they probably rested in shaded places amongst reeds or under bridges, etc., but though we often looked for them in such places

we never found them. They were always found in houses, stables, and in a well situated within twenty yards of our hut. From this well we could always secure plenty of specimens for study. Although continually looking for them and disturbing them in houses for the purpose of capture, none of us were ever bitten.

Conclusions.

This experiment has certainly proved beyond doubt that mosquitoes, and only mosquitoes, are capable of transmitting malarial fevers; that protection from their bite implies absolute immunity; that the protection is easily obtained, and does not in any way interfere with the ordinary avocations of life.

The sanitation of malarial regions is now within the pale of possibility. It implies the destruction of mosquito-breeding places in the neighbourhood of habitations by suitable drainage and cultivation, and the education of the people in the *rôle* of the mosquito, and in the prophylactic use of quinine and mosquito netting.

APPENDIX A.

HÆMOCYTOZOA OF ANIMALS.

During our sojourn in the Roman Campagna, we examined the blood of various animals in order to study their hæmocytozoa, and possibly to ascertain whether any of the local vertebrates might foster the endogenous cycle of our malaria parasites, and thus become factors in the epidemiology of the disease.

Already Celli, Sanfelice, Santori, Grassi, and Dionisi had made similar researches in the vertebrate fauna of the Roman Campagna, and had found parasites in the blood-corpuscles of frogs, larks, sparrows, pigeons, owls, oxen, and bats.

Our researches on the subject were somewhat limited on account of the short time we were able to spare for such investigations, and the difficulty we experienced in procuring the necessary material. The people of the locality were for the most part unwilling to let us examine their domestic animals, and, notwithstanding the rewards we offered, gave us no assistance in the capture of wild animals. The animals we were able to examine were captured by ourselves during our excursions. There was a great dearth of mammals and birds in the region, and the hours in which we were able to be about were certainly the least favourable for hunting. However, we managed to examine 188 reptiles, 33 amphibians, 24 birds, and 104 mammals.

Lizards.—Both *Lacerta muralis* and *Lacerta viridis* are exceedingly common in the Roman Campagna. Both offer a great variety of colouring more or less in harmony with that of their special surroundings. *L. viridis* varies in colour from a pale yellowish green with white stripes along the sides of the back, to the most intense emerald-green studded with numerous black spots. *L. muralis* is of a light tawny colour on the yellowish sands along the sea-shore, and of a glossy black colour dotted all over with deep green spots in thick jungle.

We examined 120 specimens of *L. muralis* and 25 specimens of *L. viridis*, and we found *Hæmogregarina lacertarum* in nine of the former and in one of the latter. Celli and Sanfelice,[1] in 1891, examined 100 specimens of *L. muralis* and 20 specimens of *L. viridis*, but failed to find any parasites.

The parasite found in *L. muralis* was no doubt that described by Labbé[2] and called by him *Karyolysus lacertarum*. We noticed the small endoglobular phase and the so-called vermicule phase, which may be contained

[1] A. Celli e F. Sanfelice.—"Sui parassiti del globulo rosso nell' uomo e negli animali," 'Annali di Agricoltura Roma,' 1891.

[2] A. Labbé—"Parasites endoglobulaires du sang des vertébrés," 'Arch. de zoologie expérim.,' Paris, 1894.

within the corpuscle or may lie free in the plasma. We also noticed the large oval bodies with numerous chromatoid granules at the periphery, probably representing early forms of the endogenous multiplication phase (*cytocysts*).

The early endoglobular phase consists of minute round or oval bodies, with very bright granules. These bodies enlarge within the endoplasma of the erythrocyte, and acquire an elongated cylindrical shape, with brilliant granules at the extremities. Usually one extremity tapers somewhat into a point, and gives the parasite a club-like appearance. The parasite is usually placed lengthways parallel to the long axis of the corpuscle, and lies extended; but it may bend round the nucleus, or it may double up on itself. The infected corpuscle soon becomes pale and enlarged; its nucleus is pushed on one side by the growing parasite, and as a rule becomes greatly elongated.

Of the infected specimens of *L. muralis* several had very slight infections, but in three of them there were large numbers of parasites. In a series of twenty very young lizards examined no parasites were detected. The lizards collected at Maccarese were far more frequently infected than those captured in the district of Ostia.

The definitive host of *Hæmogregarina lacertarum*, if there be one, is as yet unknown. On several lizards a small blood-red acarus very similar to *Geckobia latastii* was present, and may or may not have had some part in the causation of the infection. This parasite was usually found on the legs or between the scales of the tail.

The *Hæmogregarina* found in the blood of *L. viridis* did not differ in any apparent way from that found in *L. muralis*.

Ten geckos (*Tarentola mauritanica*) were collected in various houses and examined, but they did not contain any hæmoparasites.

Tortoises.—We also examined eight land tortoises (*Testudo græca*), which were found in the forest of Castel Fusano, but they were not infected. We were not able to obtain any specimens of the fresh-water tortoise (*Emys*

orbicularis) ; but Celli and Sanfelice, who examined several of these animals from the swamps of Ostia and Maccarese, found no parasites in any of them, although they frequently found *Hæmogregarina stepanowi* in tortoises of the same species from Pisa and Venice.

Snakes.—None of the snakes examined (*Zamenis gemonensis, Cornella austriaca, C. girondica, Vipera aspis*) contained Hæmocytozoa, nor did we find any parasites in the blood of twenty-one slowworms (*Anguis fragilis*), some of which were collected at Maccarese, others at Ostia.

Batrachians.—*Hæmogregarina ranarum* and *Hæmogregarina splendens* were both found very frequently in the edible frog (*Rana esculenta*), but no parasites were found in two specimens of *R. agilis*. Ten toads (*Bufo vulgaris*) were also examined, but no parasites were found in their blood.

Birds.—On account of the difficulty experienced in obtaining birds, few were examined.

We found *Hæmamœba danilewskyi* in two out of six pigeons (*Columba livia*) examined. The other birds examined were three jackdaws, two kingfishers, five swallows, three owls, one blackbird, and one sandpiper, but in none of them were parasites found.

Bats.—Dionisi,[1] in 1898, discovered three new hæmocytozoa in the blood of three different species of bats. The first he found in *Miniopterus schreibersii*, and is very similar to the parasite of quartan fever. The second he discovered in *Myotis myotis* (vel *Vespertilio murinus*) ; the parasite of this bat in some of its stages resembles the quartan parasite, in others that of tertian fever. The third was an unpigmented parasite analogous in some of its phases of development to the parasite of so-called æstivo-autumnal fever ; Dionisi found it in the blood of *Vesperugo noctula*.

For the classification of these parasites, Dionisi proposed two new genera, one of which he named *Polychromo-*

[1] A. Dionisi.—"La malaria di Alcune specie di pipistrelli," 'Annali d' Igiene Sperimentale,' Roma, 1899.

philus, because of the different behaviour of the parasites towards Romanowski's stain in their various stages of development; while the other he called *Achromaticus*, because the parasite belonging to this genus never contains any pigment.

To the genus *Achromaticus* belongs the parasite of *Vesperugo noctula*, to which he gave the specific name of *Achromaticus vesperuginis*, and to the genus *Polychromophilus* belong the hæmocytozoa of *Miniopterus schreibersii* and *Myotis myotis*, for which Dionisi proposed the respective specific names of *Polychromophilus melanipherus* and *P. murinus*.

We examined very carefully fifty-eight specimens of *Myotis myotis*, two specimens of *M. capaccinii*, and seventeen specimens of the lesser horseshoe bat, *Rhinolophus hipposiderus*. About twenty of the *Myotis myotis* and one of the *M. capaccinii* were captured in some tufa caves near Ariano; all the other bats were collected in the district of Ostia.

We found no parasites in the numerous specimens of *Myotis myotis* and *Rhinolophus hipposiderus*, but both specimens of *Myotis capaccinii* contained hæmocytozoa corresponding exactly to the *Polychromophilus melanipherus* described by Dionisi as the parasite of *Miniopterus schreibersii*.

Myotis capaccinii, commonly known as the hairy-tail bat, is rare in Italy; it is not found in large clusters like *M. myotis*, but as isolated individuals or amongst the clusters of *M. myotis* and *Miniopterus schreibersii*.

The two specimens of *M. capaccinii* were captured and examined in June; only one or two parasites could be seen in each slide of peripheral blood. These parasites were to all appearances gametes. They were ovoid or roundish bodies, with coarse granules of black pigment, similar to those of quartan parasites, scattered about the cytoplasm in various ways or gathered at its periphery. Some of the parasites seemed free in the plasma, others were endoglobular and occupied almost the entire erythrocyte, which was

thereby somewhat altered in shape, but not appreciably enlarged.

Post mortem many parasites were found collected in the cerebral capillaries, and there were some evidences of a slight pigmentation; no pigment was seen in the liver and spleen, and the latter organ was not enlarged.

Most of the bats were covered with ectoparasites of two sorts, namely, certain Acarina (*Pteroptus vespertilionis*, etc.) and a dipterous insect (*Nycteribia dufourii*, see Pl. XXII, fig. 2), but we were not able to ascertain whether these parasites play any part in the transmission of the infection.

Cattle.—Hæmoglobinuric fever (Texas fever) is by no means an uncommon disease of cattle in the Roman Campagna, but it is far more deadly to herds of imported cattle. The indigenous greyish-white, long-horned stock seems to have acquired a certain degree of immunity. It is only after the heavy and long-continued work of the harvest season that some of the local animals are attacked. These native cattle live in a state of semi-wildness, and have no shelter.

During our stay in Ostia, there occurred five cases of hæmoglobinuric fever, three amongst local cattle and two in imported animals. We were able to examine three of the cases; of the other two one had died and the other had recovered before any news reached us of their sickness.

Case 1 was an indigenous ox. When we called to see it, the animal was lying in a grass field, with the head close to the ground, and in a dull, listless condition, which is very appropriately pictured by the name "tristeza" given to the disease in South America. The animal had been ill for several days, and had been passing red water. It was considerably emaciated, had well-marked jaundice of the scleroticæ, and was so feeble that it was with great difficulty it could totter to its legs. The muzzle was dry and hot, and on passing the hand over the body, a high temperature was very perceptible. While we were examining

it, it passed about one pint of dark port-coloured urine. A large number of ticks were found on its hind legs, and especially round the mammæ. They belonged to different species, *i. e. Hyalomma ægyptium, Rhipicephalus sanguineus,* and *Rhipicephalus annulatus.* A careful examination of blood-films taken at the time revealed the large pear-shaped parasite (*Piroplasma bigeminum*) discovered by Theobald Smith.[1]

The ox died next day, but as the weather was exceedingly hot, and as no information was sent of its decease until the following day, a *post-mortem* examination was deemed useless.

Cases 2 and 3 were two Swiss-Lombard cows which had been recently imported by the Ravenna colonists, and were kept during the summer in Ostia chiefly to supply us with milk. Both cows developed high fever on the same day. They had been feeding in the same field, and inhabited the same byre. On the following day one of them passed redwater.

One of these cows was treated by free bleeding and copious purging, with the result that it died the same day; the other was fortunately left alone, and, though passing through a severe attack of fever with the characteristic emission of redwater, eventually recovered. The large pear-shaped parasite was found in the blood of the latter. Ticks of the species *Rhipicephalus annulatus* and *Hyalomma ægyptium* were found on both cows.

A *post-mortem* examination was made of the dead cow immediately after death, and it showed the following appearances.

The animal was well nourished. The scleroticæ were slightly jaundiced. On opening the abdomen, the peritoneum was found to be markedly jaundiced. The uterus contained a fœtus at the sixth month. The heart contained much dark clotted blood in the right side. The kidneys were slightly congested. The liver was enlarged,

[1] Theobald Smith—"Preliminary Observations on the Micro-organism of Texas Fever," 'Med. News,' December 4th, 1889.

discoloured, and congested. The spleen was enlarged, very much congested, and its pulp almost diffluent. The bladder contained about a pint of wine-red urine, although the cow had not passed any redwater during life. Examination of this urine showed hæmoglobin crystals, but no red corpuscles. Blood smears were taken from the various organs. Microscopic examination of these preparations showed in a large percentage of the red corpuscles the small coccus-like *Piroplasma*. The greatest number of infected corpuscles was found in the smears from the kidney. No parasites were found in the blood of the fœtus. The immunity of the fœtus has already been noticed by Smith and Kilborne[1] in cattle hæmoglobinuric fever, and by Bignami, Bastianelli, and Thayer in human malaria.

In the smear preparations from this case and in the blood-films from Case No. 1, we found certain large, highly refractive bodies of a very definite long ovoid or fusiform shape, with coarse granules of reddish-black pigment collected centrally or scattered throughout the endoplasm. These bodies struck one at first glance as possibly homologous to the crescentic gametes of æstivo-autumnal fever.

In stained specimens of blood from Case No. 1, we also found numerous corpuscles, two or more times the usual size, speckled with a variable number of granules deeply stained by Loeffler's methylene blue. These "punctate cells," first described by Theobald Smith, are in every way similar to the speckled corpuscles found in the oligocythæmia of patients recovering from æstivo-autumnal fever.

An interesting question in the study of cattle hæmoglobinuric fever is that of the relation of the large pear-shaped parasite of the early summer months to the small

[1] Smith, T., and Kilbourne, F. L.—"Investigations into the Nature, Causation, and Prevention of Texas or Southern Cattle Fever," 'Bulletin No. 1, Bureau of Animal Industry, U.S. Department of Agriculture,' Washington, 1893.

coccus-like form which is usually found later in the season. Are they different parasites or only different stages in the life-history of the same organism ?

Theobald Smith inclines to the latter view, because in two inoculation experiments with the blood from cases in which the large pyriform bodies were found, the inoculated animals developed first a similar attack characterised by the same large parasitic form, and later exhibited a return of fever with the small form, although they had been kept away from every possible source of infection.

These experiments are by no means convincing; on the contrary, they are very similar to those experiments made in man which have been so frequently brought forward in support of the now exploded theory of the unity and polymorphism of the malarial organisms of man. Thus, in 1889, Gualdi and Antolisis inoculated two patients intravenously with 3 c.cm. of blood from a patient suffering with quartan fever. In the first case an irregular fever appeared ten days after the inoculation, the blood showing the organisms characteristic of æstivo-autumnal fever. In the second case the inoculation was followed, in twelve days, by a mild irregular fever, the blood showing a few quartan parasites, but also, as in the former case, æstivo-autumnal parasites.

In two cases of cattle hæmoglobinuric fever we found the large pear-shaped parasite alone; in a third case we found the small parasite and not one single large form or any form of intermediate size which might suggest a connection between the two forms.

Our very limited opportunities prevented us from arriving at any definite conclusion, but it is quite possible that the two forms represent distinct species. Both forms seem to multiply by simple binary division as described by Laveran and Nicolle.[1] Now, if the two forms represented merely different stages in the life-history of the same parasite it would be difficult to understand why

[1] Laveran, A., et Nicolle, M.—'Contribution à l'étude de *Pyrosoma bigeminum*,' Soc. de Biologie, 29 juillet, 1899.

this parasite should have two distinct endogenous cycles in the same host, and why the process of multiplication should be exactly alike in both cycles.

The life-history of the small *piroplasma* of sheep and that of the large *piroplasma* of dogs lend to the dual view the support of analogy. In this connection, it is interesting to note that the cattleherds of the Roman Campagna assert that oxen often contract the fever when allowed to graze in fields previously occupied by diseased sheep.

Horses.—On the 8th of July we were called to examine a horse suffering from intermittent fever at the stable of the military-police station in Fiumicino. Two other horses in the same stable had sickened in a similar way about the 20th of June, and both had died. These horses were of Hungarian breed, and had been imported into Italy the previous year. They had been stabled at Fiumicino since the 14th or 15th of April. The horse we examined had sickened on the 26th of June, when its temperature had risen to over 40° C. The officer in charge told us that at first the temperature was irregular, but that later it became decidedly intermittent; there had been a paroxysm on the 4th of July, no fever on the 5th, and then again another mild paroxysm on the 6th. The other two horses had suffered from continuous fever, with a variation of about 1° C. At the same time some of the men in the rooms above had suffered from tertian fever notwithstanding the compulsory prophylactic use of quinine. At the time of our visit, the affected horse had no fever, but was very anæmic and languid. There were no other symptoms of disease. We prepared some blood-films and collected a number of mosquitoes (*Anopheles maculipennis*) which were resting on the walls and ceilings of the stable, and were all well gorged with blood. The stable was in a perfect sanitary condition and scrupulously clean.

On the previous year, also in June, all the five horses stabled at Fiumicino had contracted fever and had to be sent to Rome, where four of them continued ill from

twenty to forty days, and one till the following October.

In examining the blood from the Fiumicino horse, we only found one doubtful endoglobular, unpigmented, vacuolised and deeply stained parasite. The mosquitoes collected in the stable of the diseased horse revealed nothing, but some days later, in a specimen of *Anopheles maculipennis* captured in another horse stable in Ostia, we found in its stomach wall a body exactly like the one we had seen in the horse's blood.

Dogs.—Piana and Galli-Valerio [1] have described a large pear-shaped parasite (*Piroplasma bigeminum*, var. *canis*) in pointers, but we did not find any kind of hæmocytozoa in the dogs of Ostia, although we very frequently examined their blood.

APPENDIX B.

EXAMINATION OF DOGS FOR FILARIÆ.

Filaria immitis is very prevalent in the neighbourhood of Ostia. We found its free embryos in eight out of twenty-one dogs living in the district. It was almost invariably found in the older dogs, many of which showed signs of stiffness, suffered from dyspnœa, and were unwilling to follow us in our excursions.

The limitation of this widely distributed filaria to certain swampy regions led to the belief that its intermediary host might be a crustacean or a mollusc. In 1879, Bancroft believed to have discovered that the intermediary host of *F. immitis* was *Trichodectes canis*, the common dog louse, and Sonsino stated that he had found it in *Hæmatopinus pilifer*, another common ectoparasite of dogs. Later, Sonsino and Grassi incriminated *Pulex serraticeps*, the dog's flea, but further investigations obliged them to abandon this view.

In 1892, Calandruccio found in a mosquito a larval

[1] 'Moderno Zoiatro,' 1895, No. 9.

filaria, and suggested that it might possibly be *F. immitis*, but he did not make any further investigations. At last, in 1900, Grassi and Noè [1] positively established that *Anopheles maculipennis* is an efficient intermediate host of *F. immitis*. They found that the embryos, after ingestion, pass into the Malpighian tubes of the mosquito, there grow, and, after having attained their maximum stage of development possible in the mosquito, traverse the tissues of the insect and reach the labium, eventually escaping by rupture of the same into the next dog bitten.

By feeding mosquitoes of the species *A. maculipennis* on a filariated dog, and then keeping the insects alive for varying periods, we were able to follow out these changes and fully corroborate the observations made by Grassi and Noè, but the few experiments we were able to make do not justify us in accepting the inoculation hypothesis of these authors, which implies the rupture of the filariated labium along its dorsal aspect.

Laboratory-reared mosquitoes were applied to the infected dog by means of test-tubes until they had fully gorged themselves, then they were placed in large cages made of cotton netting stretched over a wooden frameing, and kept alive by feeding on the juices of fruit until ready for dissection. When required, the insects were killed, hardened, embedded in celloidin, and sectioned.

Twelve hours after feeding the young filariæ had migrated from the stomach, and were found lying in the Malpighian tubes. From this time onwards they gradually increased in size in a somewhat similar manner to the embryos of *Filaria bancrofti* amongst the thoracic muscles of *Culex pipiens*, and reached a stage in which a well-marked alimentary canal was visible. In sections, ten days after feeding, the filariæ were seen to have reached their maximum stage of development, and some had begun to migrate towards the head. This coincides with Grassi and

[1] Grassi, B., e Noè, G.—"Propagazione delle filarie del sangue exclusivamente per mezzo della puntura di peculiari zanzare," 'Rendicanti della R. Accademia dei Lincei Roma,' 1900.

Noè's experiments. The latter investigators found that the filariæ, in summer, usually leave the Malpighian tubes on about the tenth day, and reach the labium one or two days later.

In sections eleven days after feeding on an infected dog, some filariæ were still within the Malpighian tubes.

In sections of an *Anopheles maculipennis*, from a house near Ostia, another species of filaria was found lying in the thoracic muscles. Bastianelli has also found similar nematodes developing between the thoracic muscles of these anopheles. The definitive host of these filariæ is as yet unknown, but from the fact that *Anopheles maculipennis* is usually found in houses and stables, it is very likely that it may be one or other of the domestic animals.

APPENDIX C.

Rhipicephalus annulatus (Say).[1]

See Plate XXV.

Ixodes annulatus, Say. Journ. Acad. Nat. Sci. Philad., ii, p. 75, 1821.

Ixodes bovis, Riley. Bureau of Animal Industry, 1868.

Boophilus bovis, Curtice. Journal of Comp. Medicine and Veterinary Archives, July, 1891, and January, 1892.

Rhipicephalus annulatus, Neumann. Mém. Soc. Zool. France, x, pp. 407—414, 1897.

Length of full-grown and distended female about half an inch (13 mm.); width, less than one third of an inch (7·8 mm.); colour, a dull leaden hue, turning to a deep brown or vivid red in alcohol, but changing to a relatively small extent in formalin. Subelliptical in form when viewed from the dorsal or ventral aspect, broader behind than in front, with rounded anterior and posterior ex-

[1] We are indebted to Mr. R. T. Pocock, F.Z.S., for the technical description of this species.

tremities, the lateral margin showing a shallow constriction on a level with the points of insertion of the legs of the fourth pair. Dorsal surface sparsely hairy in distended specimens, somewhat closely hairy in those that are fasting; marked with three impressed longitudinal lines or grooves: a median extending from the centre to the posterior border, and one on each side passing backwards from a point just behind the head-shield or *scutellum*, also almost to the hinder border, slightly diverging from the middle line from before backwards, and often interrupted in front of the middle of its length. Ventral surface impressed with three grooves corresponding to those on the dorsal side: the median or anal groove extending from the anus, and the lateral or genital grooves from the genital orifice to the posterior end, these grooves being subparallel in the anterior half of their length, and diverging somewhat abruptly on a level with the anal aperture.

Head-shield or *scutellum* small, not sharply differentiated from the rest of the dorsal integument; about one fourth longer than wide; wrinkled laterally, but otherwise not noticeably sculptured; its sides subparallel anteriorly, abruptly converging posteriorly with the posterior border elliptically rounded; eyes small, situated on the margins of the plate at its widest part. *Capitulum*, or shield that bears the mouth-parts and rostrum, short and broad, transversely hexagonal. *Palpi* (appendages protecting the rostrum laterally) very short, long, externally angular, and inferiorly crested; apical subconical; the second and third segments wider than segment short and subcylindrical. *Rostrum* or *hypostome* short and broad, about one fourth longer than wide, armed below typically with four rows of teeth. *Legs* slender, sparsely hairy; basal segment of first at most weakly bidentate posteriorly; apical segment armed beneath with a terminal spike, preceded, except on the legs of the first pair, by a smaller spike. ♂ much smaller than ♀, only about 2 or 3 mm. long. The dorsal scute grooved as in the ♀, covering the entire body, its anterior end pro-

longed in front on each side into two horns, of which the inner is the smaller, and embraces the base of the capitulum; furnished posteriorly with eleven festoons, and in the variety named *micropla* with a median caudal prolongation. Ventral surface provided on each side of the anus with a pair of long, horny, subequal, adanal plates, which extend as far forwards as the basal segment of the fourth leg. *Legs* robust, with basal segment large; that of the first leg shortly bidentate behind, produced into a forwardly directed prominence in front.

This species is cosmopolitan in its distribution, occurring in all tropical and temperate climates to the south of about the 45th parallel of north latitude. It lives parasitically upon mammals of various kinds, and as a carrier of the parasite of cattle hæmoglobinuric fever has gained world-wide notoriety. Curtice thinks that it was introduced early in the sixteenth century into the Spanish settlements of America, and thence spread with the cattle to all such places as offered suitable surrounding conditions. Its original habitat was perhaps the Mediterranean basin. Curtice further expresses the probability that cattle hæmoglobinuric fever, which was also called Spanish cattle fever, may have been introduced at the same time from the old world with the cattle and their ticks.

Two other species of *Rhipicephalus*, of equally wide distribution in virtue of their infecting domesticated animals and following the wanderings of civilised man, may at first sight be confounded with *R. annulatus*. These are *R. sanguineus*, Latr., and *R. bursa*, Canestrini. Although closely related to one another, these two may be readily distinguished from *R. annulatus*. The palpi are relatively longer, and externally convex, instead of short and externally angular. The basal segment of the first leg is strongly and deeply bidentate in both sexes, and the male is provided with a single short adanal plate instead of the pair that are present in *R. annulatus*.

R. bursa may be distinguished from *R. sanguineus* by having the scutellum as broad as long, and coarsely punc-

tured, and by the posteriorly more widely expanded adanal plate, etc.

Yet a third species of not uncommon occurrence upon cattle and beasts of burden is *Hyalomma ægyptium*, which is distributed over the countries of the Mediterranean, Africa, India, etc. This is a larger species than either of the three species of *Rhipicephalus*, the distended ♀ reaching a length of 20 mm. or thereabouts. It also has the rostrum and palpi very much longer, and the eyes away from the margin of the scutellum. Furthermore, there are two short adanal plates on each side in the ♂ which only just surpass the anus in front, while above them on each side there is frequently a single horny prominence.

Rhipicephalus annulatus does not pass from one host to another, as was at one time believed, but completes its life upon the same host. The larvæ, when they emerge from their egg-shells, possess only six legs and, apparently, no sexual organs. They do not develop unless they be placed on a suitable host. When placed on cattle their growth begins at once, and in about a week they undergo a first ecdysis, from which they emerge with another pair of legs and a pair of large stigmata behind them. They are now *pupæ*, and go on developing until, in about another week, a second moult leads to the adult stage, with perfectly developed reproductive organs. Fertilisation then takes place, and the female begins to distend enormously with the growth of the eggs and the large amount of imbibed blood. After about three weeks of parasitic life the mature female drops off to lay her eggs amongst the herbage. The eggs number from one to two thousand, and are laid one by one, and each one is in turn coated with a glutinous protective substance, which is secreted by a pair of racemose glands situated just under and within the head-shield. Oviposition lasts a considerable time, and while the pile of eggs grows larger the body of the animal contracts, until nothing more is

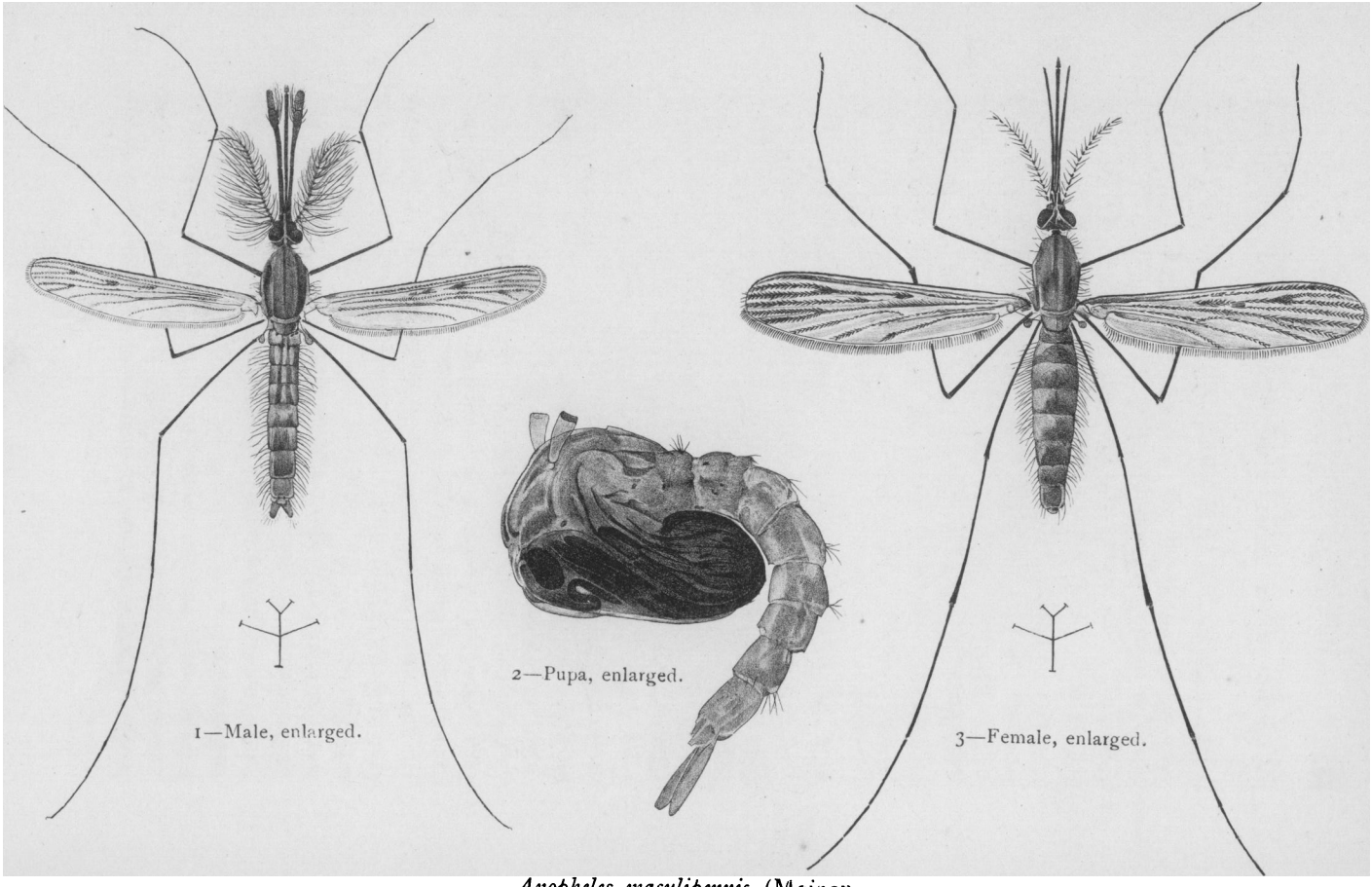
left than a yellowish, dried-up, shrivelled skin, whence all life has departed.

The eggs consist of a thin, shell-like covering, with a dark, opaque mass within. In the latter stages of incubation the form of the larval ticks becomes more and more apparent. The enormous quantity of blood imbibed by the female ticks serves for the development of their progeny; in fact, it becomes the vitellus with which the eggs are largely supplied, and residues of which may be seen within the abdomen of the newly-hatched larvæ. The most interesting feature in the experiments made by Smith and Kilborne is the demonstration that cattle hæmoglobinuric fever may be transmitted by ticks hatched in the laboratory, and that therefore the parasite must pass from the mother-tick to her progeny. This fact was at first generally discredited, but Koch repeated the experiment and confirmed it. However romantic it may seem, this fact is by no means unique; *Pebrine*, a protozoan disease of silkworms, is likewise transmitted, through the eggs, from the moth to the caterpillars.

As yet we know nothing of the exogenous cycle of *Piroplasma bigeminum*. The parasite has not been demonstrated in the salivary glands of ticks, but experiment has positively proved that it is inoculated by them. This may be explained by supposing that the parasites pass to the eggs with the vitellus which is derived from the infected blood imbibed by the fertilised female tick.

The *intra-ovum* inclusion of parasites is by no means uncommon; a trematode, *Distomum ovatum*, may be found occasionally in the white of the eggs of fowls, having been enveloped in the albumen during its excursions into the oviduct. Nematodes and other parasites have likewise been found enclosed in the eggs of fowls, ostriches, and other birds.

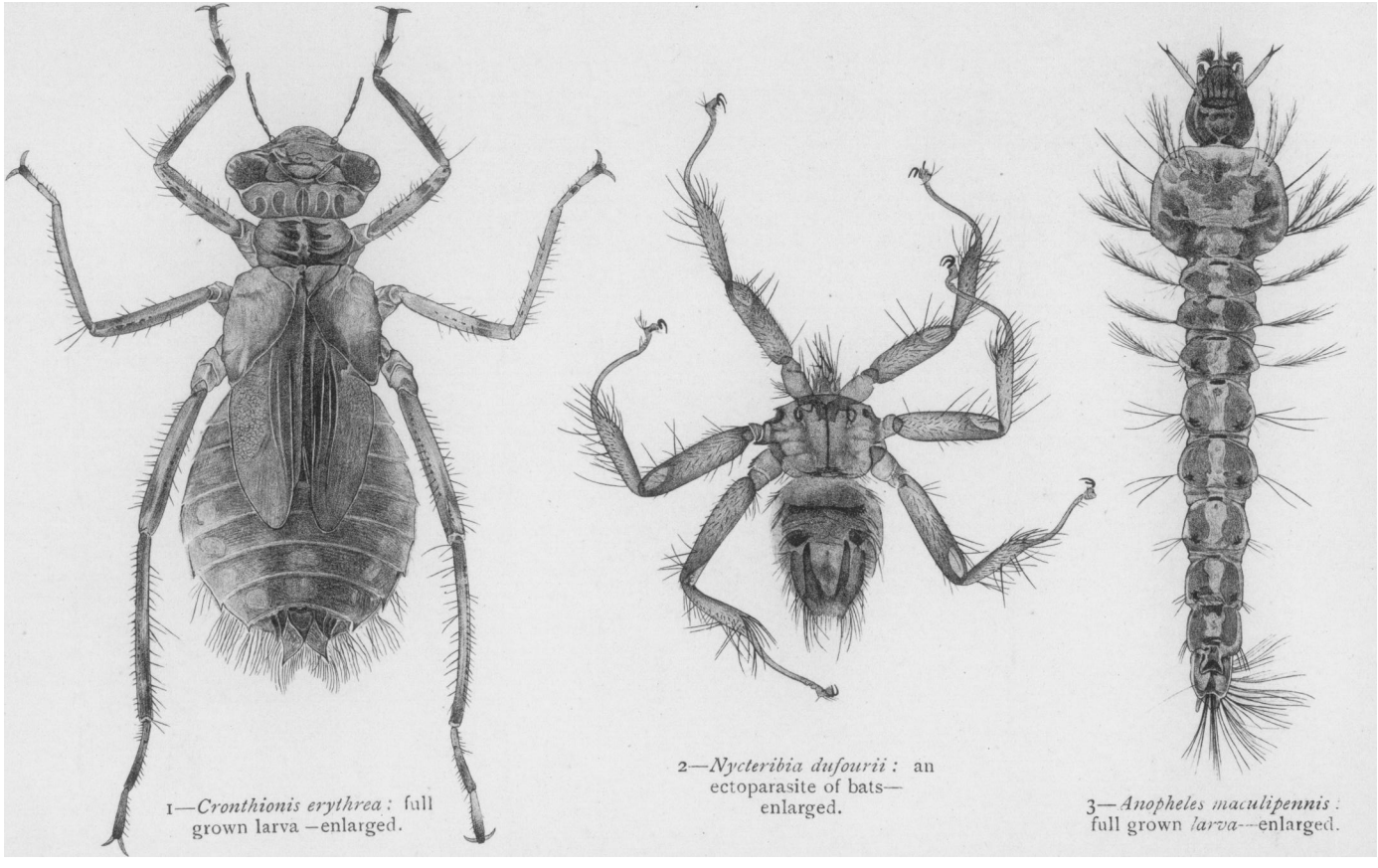
Probably the transmission of *Piroplasma bigeminum* occurs only through the fully-grown females at the time when, like the females of certain mosquitoes, they imbibe blood.



Anopheles maculipennis (Meigen).

Drawn by A. Terzi.

Bale and Danielsson, Ltd., Lith



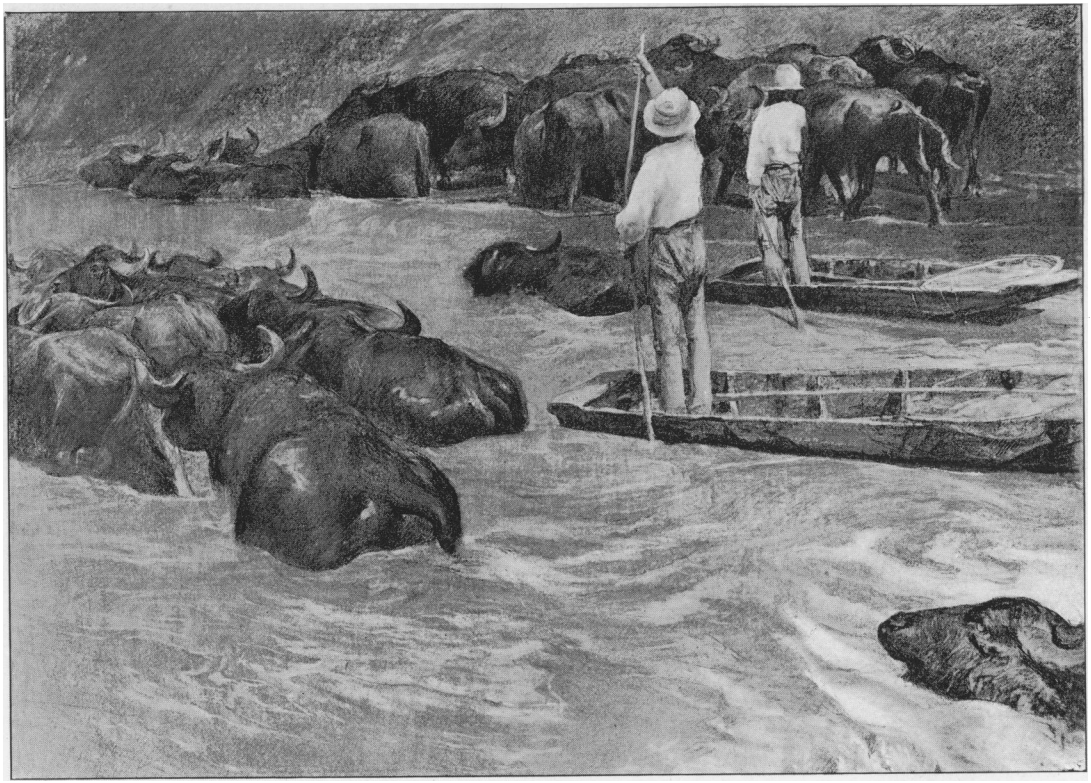
1—*Cronthionis erythrea*: full grown larva—enlarged.

2—*Nycteribia dufourii*: an ectoparasite of bats—enlarged.

3—*Anopheles maculipennis*: full grown *larva*—enlarged.

Drawn by A. Terzi.

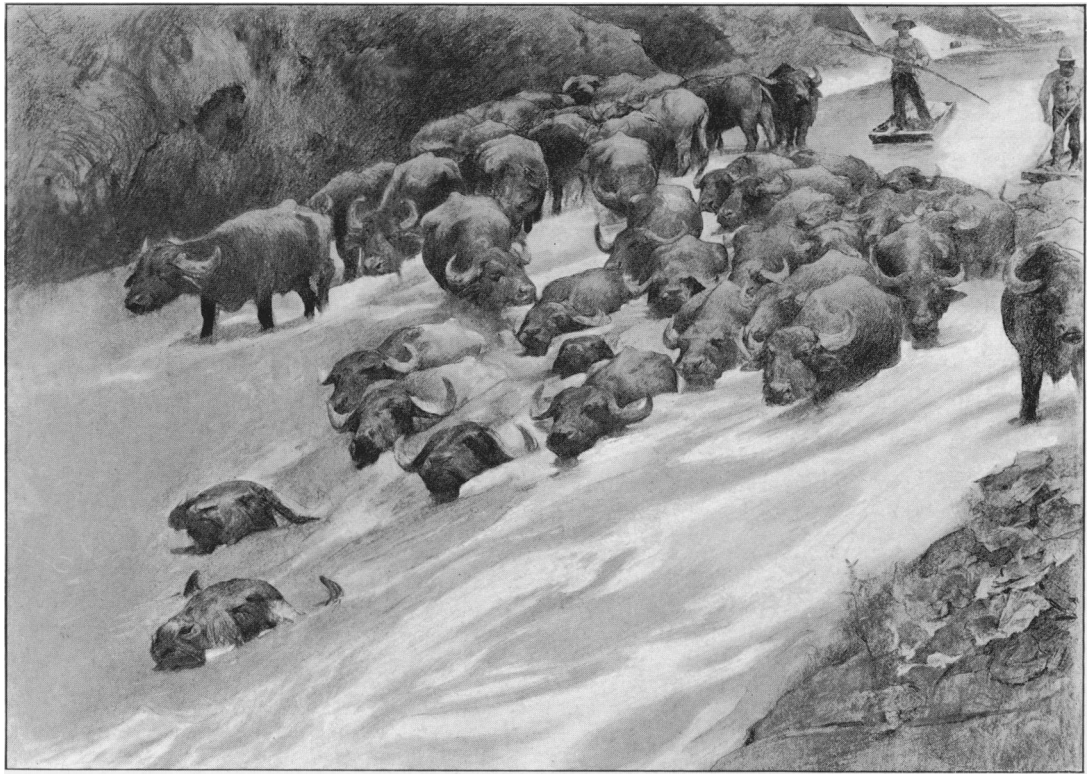
Bale and Danielsson, Ltd., Lith



Driving a Herd of Buffaloes into Irrigation Canal for the purpose of weeding it.

(Drawn by A. Sartorio.)

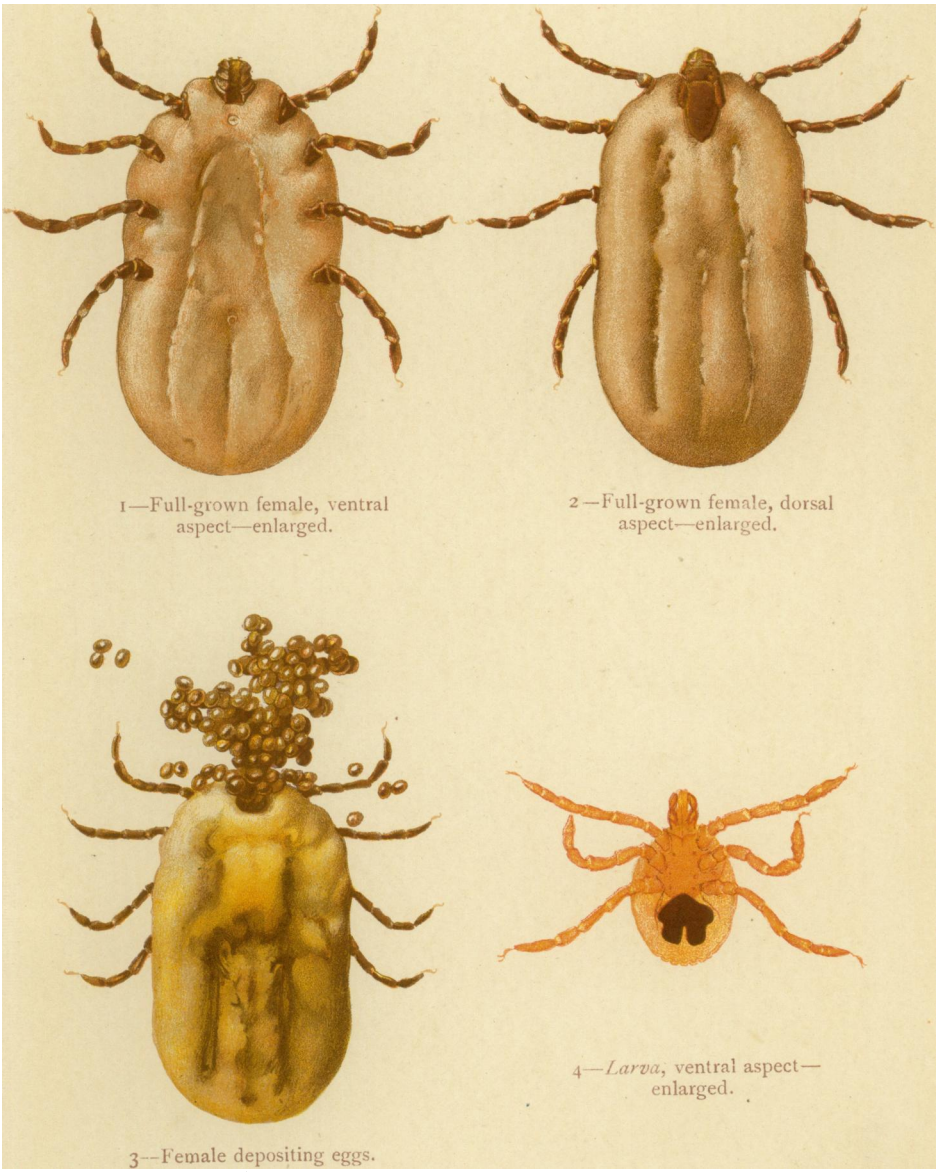
Bale and Danielsson, Ltd.



Buffaloes weeding Irrigation Canal—Pontine Marshes.

(*Drawn by A. Sartorio.*)

Bale and Danielsson, Ltd.



1—Full-grown female, ventral aspect—enlarged.

2—Full-grown female, dorsal aspect—enlarged.

3—Female depositing eggs.

4—*Larva*, ventral aspect—enlarged.

Rhipicephalus annulatus (Say).

Drawn by A. Terzi. *Bale and Danielsson, Ltd., Chromolith.*