STUDIES ON THE NATURAL HISTORY OF YELLOW FEVER IN EAST AFRICA.

With Notes on Other Insect-Borne Infections

by

A. J. HADDOW

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CONTENTS

INTRODUCTION

The writer began work on mosquitoes in East Africa in 1940 and soon found that a good deal of his interest centred on the biting behaviour of the female, a subject concerning which there was very little reliable information at that time. After nearly two years at Kisumu, Kenya, where work was devoted mainly to a study of the activities of mosquitoes in African huts, together with some observations on larvae, he joined the staff of the Yellow Fever Research Institute at Entebbe, Uganda. The Institute at that time was a field branch of the International Health Division of the Rockefeller Foundation. The main work of this unit was an investigation of the epidemiology of yellow fever in East Africa and the Belgian Congo, though studies on other viruses were also included. Here the work changed at once from indoor studies in huts to outdoor studies in rain-forest and in African plantations, but once again the biting behaviour of the female mosquito remained a most important field of study, though a considerable amount of work on larvae was also carried out. Gradually, as knowledge increased, the scope of the investigation was widened to include work on wild animals, particularly monkeys, as it became obvious that studies on the behaviour of mosquitoes, apart from that of wild animals, could never give a complete picture of the activity of yellow fever or of other viruses in nature. The observation of wild animals and the collection of animals and animal sera has played an increasing part in the investigation, till at present there is as much mammal as mosquito work in hand at Entebbe.

Much of what may be called the natural history of yellow fever in Africa still remains obscure and, since the elucidation of the main problem in the wetter forest areas in 1948, work has moved to the drier bush country and even to semi-desert. Here the same or similar lines of enquiry continue, in the complicated task of unravelling the relationships between man, the vector insects, the wild animal hosts, and the virus. The same lines of work apply also in the case of the other viruses under study at Entebbe.

From these studies has come the group of papers now submitted.

These may at first seem rather diverse in subject-matter, but almost all follow a single main line of enquiry, the relationship between animal and mosquito behaviour and human disease, more particularly yellow fever. The object of this communication is to present a narrative describing the course of the investigation and linking up these papers.

In the Yellow Fever Research Institute the entire staff worked as a team - of which the writer was the field member - and in consequence much of the work was carried out in collaboration. In such cases the extent of the writer's contribution is indicated in the list of papers submitted. Broadly speaking, it was usually the major one in field work with animals and mosquitoes, while in laboratory virus work the reverse was the case.

It should be mentioned that the Entebbe Institute is now administered by the East Africa High Commission, and that its present name is the East African Virus Research Institute.

It will be noticed that in several of the papers submitted, particularly the earlier ones, there are a number of corrections by hand. This is due to the fact that during the war and for some time afterward proofs were not sent to East Africa, and so no correction was possible. Even now some journals are reluctant to send proofs overseas, even when a guarantee is given that they will be sent back by return airmail. One of the author's latest papers, for example, contains a serious error and a serious omission which would certainly have been noted and corrected had proofs been made available.

In the course of the field investigations on yellow fever a substantial number of other viruses, most of them previously unknown, have been isolated from mosquitoes, animals and man. Several of these have proved to be of considerable importance and, in various cases, their discovery resulted in a temporary transfer of the main activities of the team to the investigation of the new agent. It is hardly possible to give an account of the yellow fever investigations without discussing these other viruses. In order to avoid breaking the narrative, however, it has seemed best to place these short notes in appendices at the end of the main paper.

In the text abbreviated scientific names have been used. The full names are given in Appendix 1.

As the main work at Kisumu consisted of behaviour studies on mosquitoes in huts, more particularly with reference to malaria, it may seem inappropriate to include it here. A summary is none the less necessary in this introduction, as many of the catching methods used later in yellow fever work were evolved at Kisumu, as were various of the ideas brought into play subsequently. The topography and climate of Kisumu have been described elsewhere (Haddow, 1942a) and in the same paper will be found the details of the work now summarised.

Almost immediately after arrival it was decided that a preliminary study should be carried out to investigate the entry of Anopheles spp. and other mosquitoes to African huts, with the object of finding at what time this took place and to what extent the common species differed from one another in this respect. At that time little was known of this subject. For example, the standard textbook on the Anophelines of the Ethiopian Region (Evans, 1938), while giving an excellent summary of the available literature, had little to say on the important subjects of entry time and biting activity. Taking the case of the most dangerous malaria vector in Africa, Anopheles gambiae, it may be noted that in the course of Evans' 29-page summary of available information, only ten lines are devoted to biting habits. In the case of a similar long summary on the almost equally important A. funestus, the subject of times of activity is dismissed in four lines, the only work quoted being that of De Meillon (1934) which refers to tents, a rather artificial environment.

Work was begun in a group of huts near the township boundary. Day catches of resting mosquitoes were made in the mornings and a series of night catches (with hourly collections from sunset till sunrise) was made in a hut baited with one sleeping African. Recording climate instruments were set up in this hut for comparison with others mounted in a Stevenson screen outside. Almost at once it became apparent that work of this type would produce a good deal of new information, but at the same time it became obvious that controlled comparative studies would not be possible, as conditions in different huts varied considerably. Further, in the average hut, rapid and thorough searching was rendered difficult by the profusion of beds, gourds, earthenware jars, etc. in dark corners. An even more serious difficulty was created by the fact that as a rule most of the roof was out of reach, and that even in the accessible parts mosquitoes were difficult to find among the sooty grass and cobwebs of the thatch. This was considered a serious drawback, as it had already been shown by Gibbins (1933) that the roof was a favourite resting-place for mosquitoes.

It was therefore decided that a standard type of hut was necessary, all parts of which should be accessible to a catcher, and which would permit the comparison of one hut with another. It was considered specially important that the hut should be of a type in which even a rapid search would yield a high proportion of the resting mosquito population. With these requirements in mind, two exactly similar round huts were built of mud and wattle with grass thatch, and two more were added later. While very similar to the type of local African hut as built by the Jaluo tribe, they differed in that a sheet of hessian was stretched across to form a ceiling, this being a little above the gap invariably left between the top of the mud wall and the grass thatch (in this type of hut the gap forms the main entry for mosquitoes). The populations of the huts consisted of small boys, whose only work was to enter them before sunset and to sleep inside, not leaving till after sunrise.

The huts proved very satisfactory in use, and the fact that they were comparable opened the way for much work that had not previously been possible. The use of standard huts with low cloth ceilings has now become common, particularly in insecticide studies. So far as the writer knows, however, these were the first standard huts to be used for mosquito work. One result of their effectiveness was that the field of investigation was widened to take in other aspects of mosquito behaviour in huts, quite apart from times of entry, and towards the end of the writer's stay at Kisumu, such work far exceeded that for which the buildings had originally been planned.

A good deal of work on microclimate was carried out in these huts. Comparison with outdoor controls showed that they had a very well-marked insulating effect. Thus they were cooler by day and warmer by night than was the outer air. Where humidity was concerned, they were more humid by day and less so by night than the outer air. Saturation deficiency was never high in the huts, and this, of course, is a matter of great importance to small insects, which are constantly in danger of desiccation should they fail to find suitable day harbourage. The most interesting application of these observations was that conditions within the huts were much more suitable for the development of malaria parasites in the mosquito than were those outside.

Mosquito work in the huts was based on long series of catches carried out both by day and by night, under close personal supervision. One of the huts was set aside as a control and, for a year, daily catches were carried out in it - six day catches and one all-night catch per week. By day this hut was empty. A single African boy entered it each day before sunset, slept there and did not leave till after sunrise.

The day catches gave information about the resting mosquito population, and also about seasonal incidence. The night catches were mainly concerned with times of entry, but also contributed to the observations on seasonal abundance. A certain amount of night work was also carried out in tents. In all cases the method was the same - an hourly collection from sunset till sunrise.

Up till that time most mosquito work in huts had been based solely on day catches, from which conclusions were drawn about night populations. One of the first points to emerge was that this method is entirely unreliable, as various species, abundant in huts by night, leave soon after feeding and are poorly represented in day catches. The main malaria vectors, Anopheles gambiae and A. funestus do rest in huts by day. On the other hand, the two commonest Culicines, Taeniorhynchus africanus and T. uniformis leave, for the most part, before sunrise. This seemed a point of considerable practical importance, as at that time these mosquitoes were believed to be important vectors of filariasis in East Africa. Recently, however, it has been shown that this is not the case (Smith, 1955). Yet other species were found to enter mainly at sunrise, purely for shelter and in such cases, of course, day catches may give an exaggerated view of their importance as biting insects in huts by night.

The resting sites chosen were of considerable interest as, while certain species apparently preferred to rest on the walls (and mainly on the lower, darker half), over 90 per cent of the A. gambiae and A. funestus rested on the ceiling. Thus in the case of an ordinary hut the high, cobwebbed thatch, with its innumerable chinks and crevices, is probably the main resting place for the important vector species. Such thatch is exceedingly difficult to search effectively and much of it is always out of reach. Thus the ordinary hut search, mainly confined to the walls, gives at best a poor idea of their prevalence. Another interesting finding in the day catches was that quite a number of mosquitoes rest on the outside of the walls, under the eaves.

The night catches revealed that at sunset there is a fairly high resting population. As darkness falls these mosquitoes leave, and shortly afterward others begin to enter. While all the common species enter throughout the night, their times of peak entry differ widely and, in fact, they form a succession. Thus the main entry of T. africanus and T. uniformis is before midnight, that of Anopheles coustani is at about midnight, and that of A. pharoensis a little later. On the other hand, in A. gambiae and A. funestus the main entry is in the later hours, and particularly the period just before dawn. This is even more marked in males than in females and for this reason it was concluded that the dawn entry peak might be made up mainly of mosquitoes entering for shelter. Later, however, during work in Uganda, it was found that biting activity also is most active in these species just before sunrise.

This was the first long series of comparable all-night mosquito catches to be carried out. Kerr, however (1933), had carried out ten night catches in the open air. His paper, unfortunately not available to the writer at Kisumu, shows that in West Africa also A. gambiae bites very late (in his work the peak occurred from 02-04 hours). In spite of this early work of Kerr's, it was widely believed that the important malaria vectors were crepuscular and one of the main results of the long series of night catches at Kisumu was to establish firmly that this is not the case.

The fact that the huts were strictly comparable allowed series of catches to be made (in all cases including both day catches and all-night catches) to compare the results obtained with different populations of occupants. It was shown, for example, that mosquitoes are attracted to a hut containing unwashed Africans in much larger numbers than to one containing an equal number of washed Africans. Anophelines (but not _Taeniorhynchus_ spp.) entered a hut containing dirty clothing much more freely than an empty hut. Catches in an empty hut (as compared with catches in the control hut) showed a relative increase of male Anophelines, but not the absolute increase claimed by Garnham (1929).

Perhaps the most interesting work of this nature was the demonstration that the larger the number of occupants in a hut, the larger the number of mosquitoes which enter to bite. After preliminary work, a final comparison was made by catching simultaneously in huts with 1, 5, 10 and 15 occupants. Taking the series of ten day catches and two night catches together, the numbers of female mosquitoes were 640, 1,200, 1,813 and 2,734 respectively. At the time, this was taken to imply that in a crowded hut there was a greater risk of contracting mosquito-borne disease. At a later date Muirhead Thomson (1951) pointed out that the number of mosquitoes did not proceed _pari passu_ with the number of inhabitants, and therefore that ten men occupying a hut each would attract more mosquitoes than ten men all in the same hut. A corollary which occurs to the writer is that in the more crowded huts the risk could actually be less, as there are fewer mosquitoes per man. In spite of this the nuisance factor of large numbers of mosquitoes in the crowded huts was very great, and the staff refused to work in an experiment to compare huts with still larger numbers of occupants. Without question, the number of simple punctures and interrupted meals was very considerable and as the transmission both of malaria and of various viruses can occur after puncture without actual engorgement, it is perhaps better to regard this matter as still _sub judice_.

In a comparison between a hut with one African and a hut with one calf (these huts were only roughly comparable, the experiment having been carried out before the standard huts were constructed), it was found that in the hut with a man Anophelines formed 94 per cent of the catch. In the calf hut Culicines formed 95 per cent of the catch. Culex antennatus was of particular interest. In five night catches 34 females of this species were taken in the hut with an African, and 3,449 in the calf hut - a ratio of over 100:1. At the time this mosquito was not considered important, but recent work in Egypt has shown it to be a very important vector of human disease (Taylor & Hurlbut, 1953 a and b; Taylor, Work, Hurlbut & Rizk, 1956). Here there is an indication that in different areas the feeding habits of C. antennatus must differ. Obviously it feeds mainly on cattle at Kisumu. In Egypt the epidemiology of certain virus infections indicate that it must bite man freely, and this deduction is supported by the results of precipitin tests on blood meals carried out by the workers just named.

Pilot experiments on the above lines were made in huts simply divided by a hessian screen and these showed that where two comparable huts cannot be made available, a subdivided hut is adequate for most work.

Little need be said of the studies on seasonal incidence. In the case of A. funestus it was shown that the single large annual rise was correlated with the main annual rise of Lake Victoria, as previously noted by Garnham (1938). In the case of A. gambiae it was found that a certain threshold rainfall value must be passed before an increase can take place. The seasonal incidence of the Taeniorhynchus spp. was not elucidated. This subject is still under study and it is still obscure.

Muirhead Thomson (1940) had shown that in Assam the breeding of an important Anopheline, A. minimus, is to a certain extent controlled by the temperature of the breeding water. This matter was studied at Kisumu, using artificial breeding-places considered to be typical for A. gambiae and A. funestus, to find whether the seasonal incidence of these mosquitoes might be influenced similarly. This did not, however, prove to be the case. The work has been reported more fully elsewhere (Haddow, 1943). While it was in progress the writer became much interested in a predatory mosquito larva, that of Culex tigripes, which, in some pools at least, was found to be controlling the larvae of A. gambiae in an effective manner. Some work was done on this species and the results have been published elsewhere (Haddow, 1942b). Similar work with other predatory larvae in Uganda is described below.

When experimental work in the Kisumu huts was drawing to a close it was felt that a considerable amount of new information about the behaviour of adult mosquites had been gained, but for some time many imperfections had been apparent. Thus, when beginning, the writer felt that previous work had been hampered by lack of directness, and that conclusions about insects active by night were being drawn almost exclusively from catches made by day. As Muirhead Thomson (1951) was to point out long afterwards "Another development in the last ten years is the increasing appreciation of the fact that, as Anopheles are mainly active at night, many of their activities can only be studied at night." The writer's object was to apply the simple and direct method of night catches, but even here the method actually used was not sufficiently straightforward. Thus in the paper on the work in the huts (Haddow, 1942a), a good deal of space is devoted to probabilities and deductions, mainly because the method of collection did not show which mosquitoes had entered a hut to bite and which had entered for shelter only. Attempts were, indeed, made to mark individual mosquitoes by staining the larvae with dyes and various attempts were made to render the blood-meal identifiable, mainly in order to investigate which of the engorged mosquitoes taken in huts had fed on the occupant and which had fed elsewhere and then entered for shelter, but this work was not successful. Further, it was obvious that Anopheline activity was not confined completely to the hours of darkness and that a good deal occurred before sunset and after sunrise, while in the case of Taeniorhynchus spp. biting might occur freely by day in suitable surroundings. A plan was therefore evolved for a series of catches which would go on throughout the whole 24 hours, and in which one assistant would catch mosquitoes as they alighted to bite a human bait, while another would collect those which alighted on the walls and ceiling. Actually it was not possible to begin these 24-hour catches at Kisumu as the writer was transferred to other work before the series could be started. At that time, of course, the window-trap designed by Muirhead Thomson (1948) was not yet available, and it was only when this simple but most important accessory to catching came into use some years later that the full complexity of Anopheline movements in huts became apparent.

At the close of the year's work in the control hut, the writer was requested by Mr. C. B. Symes, then Senior Medical Entomologist, Kenya Colony, to carry out a series of experiments with pyrethrum powder in the huts. The results indicated a more effective and prolonged action against mosquitoes than had been anticipated. The figures are of historical interest only as, shortly afterwards, work on insecticides expanded so greatly as to become almost a profession in itself. It is believed, however, that this was the first occasion on which comparable experimental and control huts were used for work of this type. The results were published in short form (Symes, McMahon & Haddow, 1942). The present writer took no part in the preparation of this paper, beyond supplying the experimental data summarised therein on pp. 371-374.

PRELIMINARY WORK ON YELLOW FEVER AT KISUMU

At this time developments of great significance were taking place in the field of yellow fever research. In particular, a very serious epidemic had just occurred in the Nuba Mountains in the Anglo-Egyptian Sudan (Kirk, 1941). The Kenya authorities were naturally most apprehensive about the situation, and all over the country precautionary measures were brought into action, considerable attention being devoted to the classical vector of the disease, Aëdes aegypti. At this time the writer was asked to undertake a survey of the mosquito fauna of huts within the half-mile strip surrounding Kisumu airfield. This proved to be a time-consuming task, as the number of huts was very large indeed, and finally a sample was chosen, the huts being examined at regular intervals. Most unexpectedly it was found that while A. aegypti larvae could be taken in a very considerable percentage of the huts, no adults could be found, and none whatever were taken biting. A similar result was reported by Mr. J. O. Harper, who was carrying out a companion survey on the Kano Plains, east of Kisumu. It may also be noted that the whole year's work in the standard huts and the numerous previous catches in other huts had not yielded a single adult of this normally "domestic" species, among approximately 50,000 mosquitoes taken. This was the writer's first experience of a situation which was to become very familiar later, widespread breeding by A. aegypti in areas where it can seldom be taken biting man, a factor of the greatest importance in any appraisal of the activity of yellow fever in East Africa.

Both Harper and the writer found that the larvae of Aëdes metallicus were exceedingly prevalent in domestic containers, while those of Aëdes simpsoni occurred occasionally. The potential importance of A. metallicus was not at that time appreciated, but later it was shown to be capable of transmitting yellow fever virus under laboratory conditions (Lewis et al., 1942). On the other hand, while these surveys were actually in progress at Kisumu, news came that yellow fever virus had been isolated from a human case in Bwamba County, in western Uganda, and that A. simpsoni had definitely been incriminated as a vector in that area, work later published by Mahaffy, Smithburn, Jacobs & Gillett (1942), and discussed more fully in the next section.

HISTORICAL NOTE ON YELLOW FEVER

As the writer's main work on yellow fever began at the end of 1941, it is desirable to outline at this stage the state of knowledge concerning the epidemiology of the infection at this time.

For centuries the disease had been recognised as one of the most serious scourges of the African West Coast, an area from which it was probably introduced into the New World by the slave ships, together with the classical vector mosquito, Aëdes aegypti. It was in West Africa that the virus was finally isolated from a rhesus monkey (Macaca mulatta), which had been inoculated with blood from a mild human case by Mahaffy & Bauer (Stokes, Bauer & Hudson, 1928). A surge of new work followed this crucial discovery in spite of the fact that, until an effective vaccine was developed years later, the case incidence and death rate among the investigators was distressingly high.

One of the first important things to be established was that the virus of the American and African diseases was one and the same, and this was done by Sawyer, Kitchen, Frobisher and Lloyd (1930). After this, work on both sides of the Atlantic was able to benefit by free collaboration and exchange of ideas and information.

That mosquitoes might be the vectors was first suggested by Carlos Finlay (1881) and the incrimination of A. aegypti followed not long after (Reed, Carroll, Agramonte and Lazear, 1900). While, however, the knowledge thus gained permitted effective control of the epidemic urban disease, detailed study of the disease agent in the mosquito was not possible till this agent had been isolated, as, till that time, work could only be carried on in the presence of clinically recognisable human cases to infect the mosquitoes and of volunteers to be infected by them. Now, however, with the discovery of a susceptible animal, the rhesus monkey, and with the isolation of the agent of the disease, more elaborate studies with mosquitoes became possible and much detail concerning the behaviour of the virus in A. aegypti was worked out. In addition, studies on other mosquitoes as vectors were undertaken, and

Bauer (1928) demonstrated that various African Culicines were capable of transmitting the virus by bite under laboratory conditions. Numerous other workers followed Bauer's lead, and the list of potential vectors, as now known, is a long one. A. aegypti, however, was for many years the only known natural vector, the first isolations of virus from naturally infected mosquitoes of this species in Africa being made by Beeuwkes & Hayne (1931).

In spite of the great strides in yellow fever investigation taken at this time, the work continued to be hampered by the fact that monkeys, which are large, difficult to handle, and expensive to purchase and maintain, were the only effective laboratory animals. Theiler's (1930) discovery that white mice were susceptible revolutionised the work as the animals are cheap, and can be bred rapidly in large numbers.

Now for the first time survey work became economically possible and study of the yellow fever position in the hinterland - hitherto almost unknown - was begun, spreading gradually further and further afield to take in first the French territories and the Belgian Congo and finally the countries of eastern and southern Africa.

It is to be noted that an attack of yellow fever (or an inoculation with yellow fever vaccine) produces in man and in susceptible mammals antibodies which persist for years - probably for life. Such protective antibodies may be detected by a neutralisation test carried out in mice, the serum concerned being tested for its ability to neutralise active yellow fever virus (Theiler, 1931). This procedure is known as the mouse protection test, and there are various different methods for carrying it out. While the details of technique are not of interest in the present connection, it may be mentioned that at the Entebbe Institute the method of Sawyer and Lloyd (1931) was used till late in 1942, after which the modification described by Smithburn (1945) was introduced. In 1955 a change was again made, and a modification of the intracerebral test first described by Theiler (1933) was introduced and is at present in use. The mouse protection test is of paramount importance in yellow fever work, not only in laboratory studies but even more in the interpretation of field surveys. The test is of high specificity, and the discovery of immune humans or animals in an area indicates that the virus has been active there (an individual exhibiting protective antibodies is usually referred to as "immune" or "protective"). Thus areas of virus activity in man and animals can be mapped out and further, study of the age-incidence of immunity will often give a clue as to whether the infection is endemic or epidemic, when it last occurred, and so on. Similar methods are used in the case of numerous other virus infections, as will appear below.

Early protection test surveys carried out by Beeuwkes and Mahaffy (1934) and by Beeuwkes, Mahaffy, Burke and Paul (1934) showed that the geographical distribution of the human infection is very wide indeed in British West Africa and in the adjoining French territories, Angola and the Belgian Congo. Findlay, Stefanopoulo, Davey and Mahaffy (1936) showed that natural infections occur in African monkeys and apes, and obtained evidence of the disease in wild primates in West Africa and the Belgian Congo. Yellow fever, however, continued to be regarded as essentially an infection characteristic of the area round the Gulf of Guinea until the surveys carried out by Sawyer and Whitman (1936) revealed the presence of immune humans in the southern Anglo-Egyptian Sudan and in Uganda, with one positive specimen each from rather limited surveys carried out in Kenya and Tanganyika. Instead of these findings being accepted locally as a warning of possible danger and as an indication of a much wider eastern distribution of the disease than had been suspected, they merely aroused scepticism as to the specificity of the mouse protection test which, though new, was already standard for survey work. Even the report of a highly suspect case from the southern Sudan by Hewer (1934) raised but little interest. In spite of this the International Health Division of the Rockefeller Foundation thought it important to establish an East African unit to investigate the epidemiology of yellow fever in that area and to attempt to find what danger there was of the virus finding its way to India and the Far East, where it was unknown. It was concluded that the dense populations and abundant Aëdes aegypti of the East might favour an epidemic of disastrous proportions should the virus be introduced, and at that time it was not known whether the virus was extending its range further into East Africa toward the coast or whether it had always been present. The results of the surveys in the Congo and the Sudan had shown that the virus can be present and active for long periods without outbreaks of clinically recognisable yellow fever and thus the absence of reported cases from East Africa could no longer be taken as implying that the virus was not active there also. The Yellow Fever Research Institute at Entebbe was therefore opened in 1936 under the directorship of Dr. A. F. Mahaffy, one of the survivors of the team which first isolated the virus in West Africa.

In the same year findings of the greatest importance were reported from South America. For some years obscure cases had been reported from sylvan areas, and it seemed certain that the connection was with the forest rather than with the house (in South America A. aegypti is strictly domestic). In 1932 a completely rural epidemic in Brazil was studied in detail and it was realised that A. aegypti could have played no part in its transmission. Studies on this outbreak led to Soper's (1936) definition of what is called "jungle yellow fever" in South America, as "yellow fever occurring in rural, jungle and fluvial zones in the absence of A. aegypti." The Entebbe unit thus began work with the advantage of knowing that yellow fever is not necessarily always a "domestic" disease carried by A. aegypti as had been believed up till that time.

Subsequent survey work in Uganda (Hughes, Jacobs and Burke, 1941) revealed that the highest incidence of immunity in man is in the Western Province, and particularly in Bwamba County, Toro District. Bwamba, which is more fully described below, is a lowland area on the Congo border, cut off from the rest of Uganda by a spur of the Ruwenzori Mountains (Mountains of the Moon). Much of the lowland part of the country is covered by dense rain-forest. More detailed work followed in this area, and revealed that the incidence of immunity was low on the mountain slopes and in the open grasslands not contiguous with the forest, but was high in lowland areas near the main forest. In addition, five Bwamba monkeys were tested and one was found to be immune, an observation whose important implications were not followed up at that time.

An intensive investigation, which was to continue for many years, now began in this area. Immunity surveys continued, blood specimens were obtained from febrile Africans for inoculation into mice, and liver specimens were obtained for histological study from Bwamba residents who had died after short illnesses (the lesions in the liver, the organ principally attacked, are very specific, in fact pathognomonic, in yellow fever). In the course of this work nine strains of the hitherto unknown virus of Bwamba fever were isolated from patients by Mahaffy (Smithburn, Mahaffy and Paul, 1941). Bwamba fever had been known for some time previously as a clinical entity, rather dengue-like in general character. Similar work in another part of western Uganda led to the first isolation of West Nile virus from a febrile patient (Smithburn, Hughes, Burke and Paul, 1940). West Nile virus is now known to be one of the commonest causes of fever among children in Egypt (Taylor, Work, Hurlbut and Rizk, 1956), and it has caused a severe outbreak of dengue-like disease in Israel (Bernkopf, Levine and Nerson, 1953). Yellow fever virus was not isolated during this work and so the final proof of its activity in East Africa was still lacking. The isolations of these other viruses showed, however, that the methods employed were reliable.

On the entomological side Harper established that in agricultural areas in Bwamba the commonest Culicine living in close contact with man and attacking him by day was not the classical yellow fever vector, <u>A. aegypti</u>, but an allied species, <u>Aëdes simpsoni</u>. Later Gibbins (1942) extended this work in Bwamba and elsewhere, and showed that <u>A. simpsoni</u>, which lives in banana and other plantations, breeding in the water retained in plant-axils, has preferences for particular types of banana, together with certain other plants. His rather limited catches of adult mosquitoes also suggested that <u>A. simpsoni</u> does not enter dwellings, and that it does not like open ground. These results are in accord with unpublished records compiled by Harper and Gillett.

While this work was in progress, the very serious outbreak of yellow fever mentioned above occurred in the Nuba Mountains in the Sudan. In this epidemic over 15,000 cases and 1,500 deaths were recorded, and it is probable that the true figures were at least twice as great (Kirk, 1941). This was the most serious yellow fever epidemic ever recorded in Africa, and its occurrence showed that the results of yellow fever protection tests had indicated at least one previously unsuspected danger-spot correctly. The possibility of an outbreak in the Nuba Mountains had, in fact, been predicted by Mahaffy, but his warning had not been acted on (private communication). During this outbreak strains of virus were isolated from patients (Mahaffy, Hughes, Smithburn and Kirk, 1941), subsequent laboratory work proving conclusively that the virus was that of yellow fever and thus that the disease was active in eastern Africa. Unfortunately no entomologist was present during the epidemic. At a later date Lewis (1943) studied the mosquitoes of the area and concluded that though A. aegypti was present, it was unlikely to have been of importance as a vector in the epidemic - a conclusion which the present writer and his colleagues feel to have been based on rather slender evidence. Lewis believed that the main vectors were Aëdes vittatus, A. furcifer, A. taylori, A. luteocephalus, A. metallicus and A. aegypti, in that order. It should, however, be mentioned that it is exceptional for more than one species to transmit from man to man in a given area at a given time, and also (as will appear below) that it is not sound practice to base ideas of the potential importance of a mosquito on its apparent prevalence in an area. At a later date, Lewis, Hughes and Mahaffy (1942) carried out successful transmission experiments with A. metallicus and A. taylori, and also confirmed that the local race of A. aegypti was capable of transmitting the virus.

Having succeeded in the Sudan, Mahaffy and his co-workers resumed their efforts to isolate virus in Bwamba. Having had no success with the methods previously employed, they now tried a new system, namely, periodic collection of blood-samples from a selected group of people. This indicated that the incidence of immunity was rising in certain areas, and that therefore the virus must be active in these places. The Uganda Government, on receipt of this information, immunised the people in a zone round Bwamba in a mass-vaccination campaign, to form a protective barrier, and certain quarantine restrictions were brought into operation. Intensive work followed in Bwamba in the suspect areas and finally resulted in the isolation of virus from a non-fatal human case in 1941. The simultaneous collection of A. simpsoni in areas known to be infected led to the isolation of two other strains from this species (Mahaffy, Smithburn, Jacobs and Gillett, 1942). Thus the presence of virus was demonstrated both in the Sudan and in Uganda, the reliability of the mouse protection test in survey work was finally vindicated, and it was proved that mosquitoes other than A. aegypti may act as vectors in Africa.

After this a most thorough vaccination campaign was carried out in Bwamba, over 30,000 people being inoculated, though the previous population estimate for the county had been only 25,000 (Dr. J. K. Hunter, private communication). It was at this stage that the writer began work in Bwamba.

In discussing the investigations which followed, it seems best to adopt a narrative form, except in the case of certain circumscribed sections of the work, namely, the studies on the mosquito fauna of plant axils and on mosquitoes of the genus Eretmapodites Theobald. In the first place, various different studies went on concurrently, and the dates of appearance of the papers concerned bear little relationship either to the sequence of events or to the development of the ideas behind the work. Secondly, this investigation in Bwamba, which continued from 1942 till 1948, led to several isolations of yellow fever virus, to the incrimination of the vector mosquito of the animal-to-animal cycle of the forests and to the elucidation of this cycle in East Africa; it led to the discovery of several hitherto unknown viruses - all isolated from mosquitoes; it included a long programme of study on the ecology and behaviour of mosquitoes, the discovery of several new species and subspecies, and a similar programme concerned with the mammals of the area, more particularly the monkeys. So far, however, no connected account has been given, though part of the investigation has been discussed by Mahaffy (1949). The writer feels that such an account may be worth while, as from start to finish, the development of the programme was based largely on studies of mosquito and animal behaviour and distribution, the observations of medical value almost all arising from lines of work laid down as a result of previous zoological investigation. It has been found most convenient to divide the work into years. Thus at the end of each year the entire staff were always gathered at Entebbe for local leave, the preparation of the Annual Report, etc. As this coincided with the driest season in Bwamba, there was almost always a lull in field work at this time and work usually began again late in January.

During the first years the leader of the team, Dr. A. F. Mahaffy, moved about Africa a great deal, with occasional visits to Bwamba. Dr. K. C. Smithburn, stationed at Entebbe, handled almost the entire laboratory side of the work and the writer, stationed in Bwamba, handled almost all the field side. The country was at that time far from well known, as the Bwamba Road had only recently been opened. This mountain road was frequently closed for weeks at a time by floods, broken bridges and landslides, and communication with Entebbe, 273 miles away, was usually difficult and often impossible. From 1942 till the end of 1946 the writer was the only European resident in Bwamba, which is a distinctly isolated area, and his visits to headquarters at Entebbe had to be few and short, as in his absence work at the Field Station was closed down. After 1946 there was increasing contact with the staff at Entebbe and, with the improvement of the road, regular exchange of material and information became possible.

While the main effort was centred in Bwamba a good deal of work was carried out in other parts of East Africa by the writer and his colleagues, but it seems best to deal with this in a separate section.

THE INVESTIGATIONS IN BWAMBA

GENERAL DESCRIPTION OF BWAMBA

Bwamba County lies in the Western Rift Valley, in the extreme west of Uganda, partly occupying the area between Lake Edward to the south and Lake Albert to the north. The Semliki River winds along the flat floor of the valley, linking these two lakes and, in this part of its course, forming the boundary between Uganda and the Belgian Congo. A large part of the Bwamba lowlands is covered by an eastern extension of the great rain-forest of the Congo (the Ituri Forest), this Uganda section being known as the Semliki Crown Forest or Semliki Forest. Bwamba is cut off from the rest of Uganda by the extensive North Spur of the Ruwenzori Mountains which rise from the eastern escarpment of the Rift. This spur, which bounds the county to the south and east, ranges in height from about 5,000-7,000 feet in the north of the forest section of Bwamba to about 14,000 feet in the south, where it joins the main massif. The lower levels of the steep slopes are covered mainly by elephant grass (<u>Pennisetum</u> sp.) and by the patchy cultivation of the Bakonjo tribe, mainly millet, beans and bananas. At a height of 4,000-7,000 feet the elephant grass gives way to a zone of dense mountain rain-forest (or cloud-forest) and above this again lies a zone of mountain bamboo (<u>Arundinaria alpina</u>). In the case of peaks of over 9,000 feet this in turn gives way to fully developed alpine vegetation. None of the snow peaks lie within the boundaries of Bwamba County, but it may be mentioned that the snow-line is at about 15,000 feet.

The Semliki Forest is typical lowland rain-forest with high closed canopy, lying at an altitude of 2,500-3,000 feet. Much of the forest has reached its climax - the ironwood consociation - in which the African ironwood, <u>Cynometra alexandri</u> is dominant, sometimes occurring in almost pure stand.

In such areas the canopy is of a remarkably even height, the crowns of the ironwood trees forming a single clearly defined and very level stratum, with very few emergent trees. As has been pointed out by

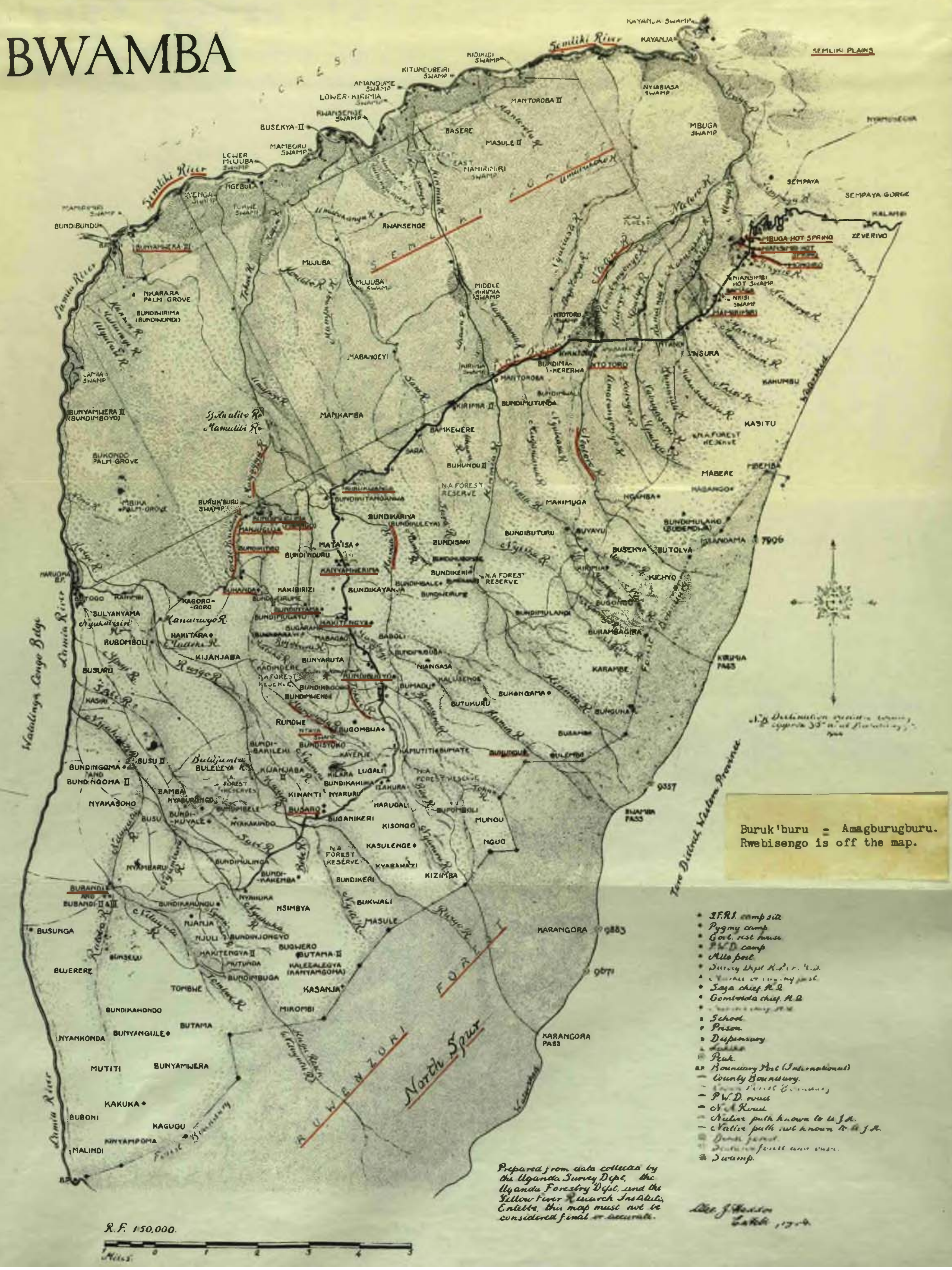
Richards (1952) this type of structure is very characteristic of tropical rain-forest where a single species is dominant. Apart from the wide areas of ironwood forest, there are also considerable stretches of swamp-forest, particularly along the Semliki River and its numerous winding tributaries. Some of these swampy areas are occupied by thickets of wild date palm (Phoenix reclinata) or oil palm (Elaeis guineensis) with a jungly undergrowth of climbing rattans (Oncocalamus sp.) and Marantaceous and Zingiberaceous plants, others by screw-pine (Pandanus chiliocarpus) growing in almost pure stand, and others - the most important in the present context - by mixed swamp-forest dominated by Mitragyna stipulosa, a large-leaved soft-wooded tree. Generally speaking the forest is very flat and exceedingly liable to flooding during the rains, the small streams pursuing very tortuous courses, which change frequently. This forest has an exceptionally rich fauna of large and small mammals, birds, insects, etc., the affinities being very definitely western. The Semliki Forest forms, in fact, the most easterly continuous part of the Lower Guinean Forest District (as defined by Chapin, 1923). This zoogeographical district extends from southern Nigeria and the Cameroons through part of French Equatorial Africa, and embraces almost the whole of the northern Belgian Congo, ending in some of the isolated rain-forests of Uganda. At present the Semliki Forest is a closed reserve, but in the past parts of it have been inhabited, and in consequence there are numerous small areas of second-growth and low thicket. The extent of the forest reserve is about eighty square miles.

In the northern part of Bwamba the forest reaches the foothills of the North Spur, and here there are connections - through forested river valleys - with the mountain forest. Further to the south, however, a wide strip of very fertile agricultural land lies between the mountain slopes and the lowland forest. This area is cultivated by the Babwizi and the Baburaburi tribes (collectively known as the Baamba), and there are also some Bambute pygmies. It is important to note that though the area is essentially agricultural, it contains much forest. In this zone between the mountain and the main forest the streams run in exceedingly deep and steep-walled valleys in which agriculture is difficult. In such valleys, as a consequence, forest tends to persist, though elsewhere, except where preserved, it is continually being cut out and burned to make room for wasteful shifting cultivation. Forest strips therefore extend far down the mountain valleys into the lowlands and similarly the lowland forest sends out numerous gallery extensions far into the cultivated area. Thus the whole of the inhabited lowland area is much intersected by belts and patches of forest and second-growth, many of them continuous with the main Semliki Forest. The main crops are bananas, hill rice, cassava, sweet potatoes, colocasia (taro), maize, beans, groundnuts, coffee and cotton. The lowlands are hot and humid, with a well-distributed rainfall of about 60 inches per year, there being no very prolonged dry season. A month without rainfall would be quite exceptional. The outstanding character of the area is the peculiarly close relationship between the people and their crops on the one hand and the forest with its abundant wild animals on the other.

Most of the work in Bwamba was carried out in this agricultural area and in the main Semliki Forest, with occasional visits to the mountain slopes and to the Ruwenzori Forest. There is, however, another part of Bwamba, lying to the north of the forest. This area, the Semliki Plains, consists of open grassland with sparse acacia woodland and reed-swamp areas. It stretches away to the southern shores of Lake Albert, and is a well-known game area, with typical East African "plains game". It is thus entirely different from the wet forest area to the south, with its essentially western fauna. Though politically part of Bwamba the plains thus form a completely separate ecological unit, and one which was of little importance in the investigations here discussed.

From the above it will have been realised that Bwamba is a remarkably varied area. In the north, the grasslands are only a little over 2,000 feet in altitude. Further south comes the main forest at a height of 2,500-3,500 feet. Last comes the mountain range with its specialised vegetation zones (the remotest point of Bwamba is now known to be the middle Portal peak of Ruwenzori, about 14,000 feet in height, which was first climbed in 1945 by a party led by the writer). The fauna and flora are corresponding-

Fig. 1: Map of part of Bwamba. This 1944 edition was the last complete map produced at the Institute. There are very many subsequent additions and corrections, but these are being added gradually to the new air survey of the county which is not yet complete. The map given here is that used during most of the investigation. Places named in the text are underlined in red.



ly varied - sometimes embarrassingly so. The mosquito list, for example, stood at 160 forms in 1948, of which 18 were first described from Bwamba material (at that time the total list for Kenya, Uganda, Tanganyika and Zanzibar stood at 269 forms) and the mammal and bird faunas are also remarkably rich, many species occurring which are found nowhere else in East Africa.

For a general view of the area in which work was carried out, reference should be made to the map (Fig. 1). A fuller account of Bwamba and its vegetation will be found in a paper by the writer (Haddow, 1945a).

THE WORKING PLAN AND THE PREPARATION OF A MAP

Field work was begun in March, 1942, having been preceded by a period at Entebbe during which a tentative programme was drawn up. It was decided that the work undertaken should include further study of A. simpsoni, particularly its breeding and biting habits and its local distribution, observations on forest mosquitoes, and the collection of wild animals - particularly monkeys - for experimental infection at Entebbe with yellow fever virus. It was considered important that further attempts should be made to isolate yellow fever virus, and that generally the trend of the work should be directed toward an elucidation of the natural history of yellow fever in the area. As has been mentioned above, it was already known that in South America outbreaks of yellow fever might occur among the monkeys in rain-forest and that man might acquire jungle yellow fever in the forest in the absence of A. aegypti (Soper, 1933, 1936). It was not yet known, however, whether a similar situation occurred in Africa, though it had already been shown that mosquitoes other than A. aegypti might be involved in transmission, certainly in Bwamba, and perhaps in the Sudan. It was also known that wild monkeys might become infected (Findlay et al., 1936), but it was not yet known whether they were important in the epidemiology in Africa. Most important of all, it was not known whether the virus could maintain itself in the absence of man (as, for example, in the interior of the Semliki Forest) or in areas where man had been eliminated from the picture by mass vaccination campaigns, as in the inhabited parts of the Bwamba lowlands.

When work was begun it was at once realised that the available maps were exceedingly inaccurate and in fact so misleading that for practical purposes they were valueless, particularly where the interior of the main forest was concerned. Most of this had never been visited, much less surveyed. A good deal of time was therefore spent in walking all over the county, collecting place names, plotting zones of forest by compass traverse, etc. The map finally produced went through many editions, not reaching its final form till 1944 (Fig. 1). Though still inaccurate, it was adequate for most of the work in hand, and even the first edition proved useful. The main forest was by far the most difficult part to prepare and the map of this area could probably never have been completed but for the help of the Forest Department. As it was, sixteen long foot journeys had to be made to plot in even a skeleton map of the forest and of the forest reaches of the Semliki River.

It should be mentioned that it had not been possible to plot accurately the results of previous immunity surveys, as most of the localities concerned were known only by name and were not marked on the existing maps. With the preparation of the new map it now became possible to fill these in and, as the various tongues and patches of forest were now shown, their relationship to forest became clear, an important clue to epidemiology becoming apparent. It had been realised that most of the yellow fever occurred in the lowlands, in areas adjacent to the forest (Mahaffy, Smithburn, Jacobs and Gillett, 1942) but, while some of the localities concerned were obviously very close to the main forest others, where the incidence of immunity was equally high, were often apparently quite some distance from it. Even the first edition of the new map showed, however, that in such cases the area concerned was almost always contiguous with one of those tongues of forest which reach out from the main block -sometimes for miles - into the cultivated country. Even more important was the fact that immunity in children - indicating recent activity of the virus - was virtually confined to localities adjacent to the main forest or to one of these extensions. With this information to hand it seemed reasonable to conclude that, as yellow fever seemed to be coming from the main forest (which was uninhabited), an animal host might well be playing a part in maintaining the disease.

It was decided that work should at first be confined to a single area, accessible from the Field Station, which had been built at Bundibugyo, on the main road. While exploratory journeys and trial catches might be made in other parts of the county, it was felt that large-scale catches and routine work should be carried out in the area chosen until the African staff had been thoroughly trained in methods of collecting adult and larval mosquitoes, and also in methods of mammal collection. It was further felt that in this initial period the writer would have a chance to familiarise himself with the local mosquitoes and the mammal fauna.

The main requirements for this first study area seemed to be as follows: It obviously should be in a locality contiguous with the main forest and preferably intersected by extensions of the forest. It should have a large human population with a known high incidence of immunity to yellow fever, not only in adults but also in children. Animals, particularly monkeys, should be abundant. There should also be abundant banana plantations, supporting a large population of A. simpsoni.

After most of the accessible lowland area had been visited, with these points in mind, an area was selected, bounded by the Bubande-Bubukwanga section of the forest boundary, by the main road and by the forested valley of the Manjuguja River (Fig. 1). Through the middle of this area the deep steep-walled valley of the Tokwe River (also heavily forested in much of its length) runs to the main forest. Over the whole area plantations and small patches and strips of forest are mingled and, though there is a dense human population, large and small mammals of many kinds are abundant. Elephant and buffalo raid the plantations, leopards are common, and the Primate fauna is abundant and varied. In past work the inhabitants of this part of the lowlands had shown a high incidence of immunity to yellow fever, and immune children had been found. Further, the virus had been isolated from A. simpsoni caught within its limits. It was also very conveniently placed from the point of view of accessibility. Here work was begun in March, 1942.

WORK ON MOSQUITO BREEDING IN PLANT AXILS

The first entomological work begun in Bwamba in 1942 was a study of plant axils and their fauna of mosquito larvae, as plant axils are the principal breeding-place of A. simpsoni in Uganda. Preliminary work on axils had already been carried out by Gibbins, but only his main findings were available till the end of the first year, when the published work appeared (Gibbins, 1942). It was known, however, that the plants he had found important were pineapples, colocasias, and a variety of banana known as "gonja", and this observation was taken as a starting point. It should be explained that the numerous types of banana grown in Uganda play a large part in the national economy, forming the staple diet of several of the more important tribes.

It was felt that detailed studies on these and other plants must be undertaken and that mosquito output, seasonal incidence of the larvae and adults and the distribution of A. simpsoni breeding-places in Bwamba must be investigated in some detail. Further, as Dunn (1926, 1927b) had shown that tree-holes are the preferred breeding-place of this species in West Africa, it was felt that the possibility that plant-axils might not be the only breeding-places in Bwamba should also be investigated. The relationship of adult populations and foci of breeding to the main forest was another important subject calling for study.

The work now begun was spread over a number of years and was not published till much later (Haddow, 1948). As it was a part of the programme which proceeded independently and which was not influenced by the virus investigations in adult mosquitoes, it is convenient to treat it here as a whole. Work on adult A. simpsoni will be discussed in a later section.

In Bwamba various plants occur whose axils are capable of retaining enough water to permit larvae to develop. Among these the bananas will be discussed first. The wild banana is, within Bwamba, confined to the high grasslands and the lower border of the Ruwenzori Forest, at heights above 5,000 feet. It is an important focus of mosquito breeding. The leaves are enormous - up to 15 feet long - and the axils are correspondingly large, not infrequently holding a litre or more of water. The small fruits are not used as food locally. At the time when the above-mentioned paper was written, this plant was placed as Musa sp.aff.ensete. Since then, however, the wild bananas have been assigned to another genus, and the correct name is now thought to be Ensete edule. The cultivated bananas are of much more importance, and here there are three groups to be considered, known locally as "bitoke", "menvu" (or "kasukali"), and "gonja". Formerly it was held that cooking bananas, often known as plantains, should be assigned to Musa paradisiaca and that they were distinct from the sweet eating and beer bananas which were placed under M. sapientum. When the author's paper was written, it had already been recognised that this grouping was not sound, none of the characters being constant, at least in Uganda (Thomas, in Tothill, 1940), and all types were referred to simply as bananas, Musa (Eumusa) spp.indet. Since then, however, a more authoritative pronouncement has been made by Baker and Simmonds (1949). These workers point out that all the East African bananas are derived from two wild seed-bearing species of the Indo-Malayan region. From one of these, M. balbisiana, the gonjas are derived, while from the other, M. acuminata, come the other types here mentioned. This seems a most rational division and it is one of importance where mosquitoes are concerned. The bitoke group which, cooked unripe, forms one of the principal foods of many tribes, forms the largest section, but has proved to be of little or no importance where mosquito breeding is concerned. This is due to the fact that the side walls of the axil grip the main stem loosely, being a little everted at the edges. Thus few of the axils are capable of retaining water and still fewer of retaining enough to permit larval and pupal development. In addition, the petiole of the leaf forms a very poorly-developed gutter, and does not form an efficient mechanism for conducting water down to the axil from the large surface of the leaf. Much the same is true of the yellow-skinned and rufous-skinned sweet bananas known collectively as menvu or kasukali (that is to say "sugary"), which are extensively grown for food and beer-making, and these also are of minor importance where mosquitoes are concerned. In the case of the gonja, however, the only derivative of

M. balbisiana, the leaves arise in a close spiral, the large axils gripping the main stem closely. Most of them are capable of retaining water in adequate amounts for mosquito breeding for considerable periods, even in dry weather. In addition, the gonja petiole has its edges inrolled to form an almost closed gutter through which rain or dew is conducted down from the enormous collecting surface of the leaf to the well-shaded axil. In consequence, the gonja is a very important habitat for the larvae of axil-breeding mosquitoes both in Bwamba and elsewhere. As the fruits are used both as food and in beer-making, this type is very popular in Bwamba, and gonjas occur in almost every plantation.

Pineapples, widely grown among the other small plants in plantations, are also important, the main breeding places being the younger axils and particularly the deep cup of leaves which terminates each stem. The axils never hold large quantities of water, but are tight, well-formed and shady, and so constitute an excellent habitat for larvae.

The most important plants of all are the colocasias, known in other countries as taro or cocoa yams. Various types are cultivated in Bwamba. On the mountain slopes Colocasia esculentum is prevalent. This plant has well-formed axils, much favoured by mosquitoes. It forms a dense growth, usually attaining about 3-4 feet, and the huge peltate leaves shade the axils from direct sunshine. As the petioles spring from just above ground level, this plant is easy to study and is ideal for survey purposes. The number of larvae to be found in a plantation of this colocasia is very considerable indeed. In the lowlands a different, larger species, Xanthosoma sagittifolium, is cultivated. Commonly grown in patches in the open, it is still commoner in the shade of banana plantations, where it forms a dense undergrowth three to five feet high. The hastate leaves are often as much as three feet long (even five feet in very old plants) and give a dense shade. Two main varieties are grown in the lowlands. The first, with dark blue-green petioles, is not of much importance, as the axils are loosely formed and do not retain water well. The other, with fresh grass-green petioles, has very well-formed axils and, though the quantity of water they hold is moderate, this is the most important plant of all in relation to A. simpsoni. The underground bulbs are much favoured as food in Bwamba and, as the plant grows rapidly from root-cuttings, giving two and sometimes three crops in a year, it is to be found in almost every plantation in the county.

In the colocasias generally the large leaves droop outward at an angle to the petioles, and so do not form collecting surfaces for the axils. The petioles themselves are not helpful in this respect as they are rounded in section for most of their length and proximally have a little rim round the axil which actually prevents water from draining down into it. An axil, therefore, must depend mainly on run-off from the underside of the petiole above, and this seems a very poor source. Nevertheless, the axils contain water at all times, except in the very driest weather, often forming effective breeding foci long after other plants have dried out. So constantly are they full that Buxton and Hopkins (1927), working in the South Seas area, were led to wonder whether secretion might play a part. This question was investigated in Bwamba by planting two colocasias in boxes, and putting one in the garden, while the other remained under the verandah of the house. All water was then pipetted from the axils. The axils of the plant on the verandah remained quite dry. The axils of the other filled with the first shower and thereafter retained a normal water content.

The same workers had found traces of chloride in colocasia axils during their studies in Polynesia and Melanesia (loc.cit.), but concluded that this might be due to slow concentration of wind-borne spray from the sea. This seems to have been the case, as water from axils in Bwamba gave no precipitate with silver nitrate. It is difficult, in fact, to conjecture just where the male axil-breeding mosquito obtains its supply of chloride. The female takes up much with her blood meals, and perhaps enough is stored in the egg to carry the larvae and pupae through to maturity. Whereas, however, the new generation of females can now obtain further chloride from their blood meals, the male can get but little from the plant juices on which he feeds.

None of the remaining plants is of importance in relation to A. simpsoni in Bwamba, though one of them, Pandanus chiliocarpus, is a prolific source of other species. This plant has well-formed axils which hold a good deal of water and which harbour large numbers of larvae. The long sword-shaped leaves with their spiny edges and midrib, the spiny stems, tangled spiny prop-roots and the soft mud of the environment all combine to make this the most difficult of plants to study. It may be mentioned that in Bwamba Pandanus does not occur outside the main forest.

Sansevieria spp. (bowstring hemp) grow wild in the forest and are also cultivated here and there in small patches, for the fibre. In Bwamba the axils are usually so choked with earth and debris as to be useless for mosquito breeding. In other areas, however, these plants may be important. Thus in the hot semi-desert country of north Karamoja (near the point where Kenya, Uganda and the Sudan meet) the writer has found S. abyssinica to be a prolific source of A. simpsoni and also of Eretmapodites sylvestris v.conchobius. Here these plants may retain water for as much as a month after the last shower, in spite of the very arid climate. It is interesting to note that Hoogstraal and Knight (1951) consider E. sylvestris v.conchobius to be a potential virus vector in the southern Sudan.

Two species of dracaena, or dragon-tree, occur in Bwamba lowlands. The first is referred to by Gibbins (1942) and the author (1948) as Dracaena ugandensis. It has now, however, been assigned to D. fragrans and will be given this name in the present communication. In Bwamba this plant, used in hedging plantations, is not very important. There are numerous leaves on each stem, and the stems of a given plant may be as many as a dozen. They are slender, however, and sway with even a slight breeze, spilling water from the small axils. It is true that each stem has a deep terminal cup of leaves where a good deal of water is retained, but this habitat is dominated by the predatory larvae of Eretmapodites spp. (this predatory habit is more fully discussed in the next section). The other species, D. steudneri, is a large branching plant, often thirty or more feet in height. The branches terminate in large bunches of leaves of rather palm-like aspect, whose axils hold water well. Gibbins considered, however, that the exudation of latex renders the water in the axils toxic to mosquito larvae, a fact confirmed during the work now discussed. In the Ruwenzori Forest D. afromontana occurs in small numbers. Garnham, Harper and Highton (1946) found this plant to be of importance as a source of larvae in the Kaimosi Forest, Kenya, but in Bwamba it is too scarce to be of much significance.

In Bwamba and in many other parts of Uganda Canna orientalis has established itself as a "garden escape". Large-flowered hybrid cannas are also grown in gardens. The cup formed by the terminal rolled leaf holds water quite well, and occasionally larvae may be found in it, but it is not known whether this water persists long enough to permit full development.

All these plants are illustrated in the writer's paper mentioned above, which also gives illustrations of axil structure in bananas and Xanthosoma. The photographs also give a reasonably good impression of the agricultural area of Bwamba.

The first survey work was undertaken in the study area mentioned above, in six mixed plantations near the field station at Bundibugyo. A total of 600 plants was examined, including 100 plants each of gonja, menvu, bitoke and colocasia (Xanthosoma), and 100 stems each of pineapple and D. fragrans. The results confirmed Gibbins' view that gonja, colocasia and pineapple were the important plants. Altogether 549 larvae were taken, of which 511 (or 93 per cent) were A. simpsoni. The remaining 7 per cent consisted of four other species, including A. aegypti, of which 11 larvae were recovered and reared out for confirmation. Some idea of the relative importance of the plants concerned may be gained by computing the percentage of the total sample yielded by each, as follows:

Plant	Number of larvae taken	The same, as percentage of the total
X. sagittifolium	336	61%
Pineapple	103	19%
Gonja	99	18%
D. fragrans	7	2%
Menvu	4	
Bitoke	0	

By the time that this survey had been completed, it was realised that the entire plant was not a satisfactory unit for comparative purposes, as the number of axils might vary from under a dozen in colocasias to almost 100 per stem in dracaenas and pineapples. It was decided that work based on comparable numbers of axils would be sounder. Thus in the present survey, though the number of plants was the same, the number of axils examined varied from a total of 480 in the colocasias to 5,224 in the dracaena sample. If the results are standardised to give an "expected" number of larvae per 1,000 axils, the following results are obtained:

Plant	Larvae per 1,000 axils	The same, as percentage of the total
X. sagittifolium	700	79%
Gonja	148	17%
Pineapple	32	)
Menvu	5	) — 4%
D. fragrans	1	)
Bitoke	0 ?	)

It will be noted that gonja now moves up to second place of importance, and that the true value of colocasia, as shown by this rough but improved method, is now very high. The other plants, even when grouped, contribute very little to the total. The proportional method is, of course, not a fair one in the case of bitoke as the fact that no larvae were found in the 649 axils examined does not imply that none would have been found had more been searched. As a rough guide, however, the figures above are helpful. In Gibbins' work (loc.cit.) he had found gonja to yield slightly more larvae than did the colocasias. This is probably because he did not distinguish between Xanthosoma and Colocasia, nor between the variety of Xanthosoma with green stems and that with blue-green stems. Further, much of his comparison is simply on a basis of "percentage of plants with larvae", which is a quite inadequate basis for quantitative work. It now appeared that the important points for future work would be:

a. The number of axils examined.
b. The number and percentage holding water.
c. The number and percentage harbouring larvae.
d. The percentage of available axils (i.e., water-containing axils) which were inhabited.

The last figure turned out to be an important one, as it gave an indication of what might be termed the "breeding pressure" or relative preference of the various mosquitoes for particular kinds of plant. Thus in the present survey, if colocasia and gonja are compared, it is found that in the gonja sample there were 237 axils with water, while the colocasias had rather fewer, 215. On the other hand, in the gonja, only 27 per cent of these potential foci were colonised, whereas in the colocasia the figure was 63 per cent. This difference is reflected in the fact that only 99 larvae were obtained from the gonja, as opposed to 336 from the colocasia. Unfortunately, before it was realised that the axil was a very much more satisfactory unit than the plant, some other surveys had been begun on the basis of entire plants, and in such cases it seemed undesirable to change the method part-way through.

Records were kept of the size of the plants and of their positions in the plantations, and from these notes some conclusions were drawn. Among gonjas, the small plants growing in the shade of the larger ones were the most favoured. In pineapples the size of the plant was unimportant as here growth increases the number rather than the size of the axils and in any case only the younger axils on each stem and the terminal cups hold water really efficiently, but the plants round the edges of the plantations were more heavily colonised than those in the centre. In colocasias large plants were the best, particularly those near the edges of the plantations. The general picture was thus of very active breeding going on in the large colocasias, small gonjas and the pineapples, mainly in the shade of the large banana plants, but mainly round the edges of the plantations. As will be shown below, the plantation edges are also the habitat in which the <u>A. simpsoni</u> adult bites most freely.

It is emphasized that, as plants differ in prevalence, number of axils, amount of water retained, rate of growth and number of crops per year - to name only a few of many variables - quantitative comparison with reference to mosquito output is very difficult. The comparison of samples of equal numbers of axils is, though admittedly crude, probably the best method for field work.

It should be mentioned that the other mosquito larvae taken during this survey were Harpagomyia taeniarostris, Uranotaenia ornata v.musarum, A. aegypti and Eretmapodites ferox, a species described by the writer (see next section). E. ferox larvae are very active predators, and it was found that in axil survey work the larvae collected must be killed on the spot, or else - if they were required for rearing out - all the Eretmapodites larvae must be picked out and kept separately. Thus in the above survey the results for D. fragrans were vitiated before the larvae even reached the laboratory. The sample yielded 6 E. ferox larvae and about 20 others, all thought to have been A. simpsoni. All but one of these were eaten before reaching the laboratory (i.e., within about two hours).

A most important point which emerged during the survey was that with such a limited and specialised fauna it was possible to identify even the smallest larvae, and also the pupae. The results were thus very complete. Pupae were always counted in with the larvae. They were usually bred out, but any which died were identified. In the case of important and unusual records, breeding out was generally the method used, as in the case of the A. aegypti mentioned above.

This first survey having confirmed that colocasias and gonjas were the important plants, routine work was thereafter confined to them, and particularly to the colocasias, which are very easy to examine thoroughly. Pineapples, though they do produce quite large numbers of larvae when carefully searched, tend to form spiny thickets which make thorough examination difficult.

While it seemed likely that A. simpsoni was sufficiently prevalent throughout the inhabited lowlands to act as a vector of disease, the question now arose as to whether it might be more prevalent in forest-edge localities. If this proved to be the case then conceivably the higher incidence of immune humans in such areas might be connected only incidentally with the forest and might simply be due to more efficient transmission resulting from the presence of a larger population of vectors. To investigate this question a second survey was now made in three long strips of lowland country. As it was begun before the results of the first survey had been worked out it also was based on numbers of plants rather than of axils, though naturally the numbers of axils with water and with larvae were also recorded. In this survey equal numbers of colocasias and gonjas were examined, but the results may be considered collectively. In all 500 plants were examined, a considerable number of plantations being sampled, as follows:

a. Five miles of road in open, cultivated country, with only one strip of forest; 928 larvae recovered from 150 plants; 725 A. simpsoni (78 per cent). Expected yield of A. simpsoni larvae per thousand axils - 946.

b. Three miles of native footpath in mixed country with open ridges, forested valleys, and much shifting cultivation; 358 larvae recovered from 150 plants; 184 A. simpsoni (51 per cent). Expected yield of A. simpsoni larvae per thousand axils - 250.

c. Five miles of native footpath actually on the boundary of the Semliki Forest; cultivation and second-growth on one hand, virgin forest on the other; 329 larvae recovered from 200 plants; 132 A. simpsoni (40 per cent). Expected yield of A. simpsoni larvae per thousand axils - 143.

Thus in another very large sample, covering various types of lowland country, A. simpsoni was once more by far the most prevalent species, totalling 1,041 (64 per cent) in a sample of 1,615 larvae. In this survey the species list was extended, larvae of the E. chrysogaster group and of E. leucopus ssp. productus being taken. The former are referred to a group since, up till the present, neither the larvae nor the females can be distinguished specifically. It was now obvious that while A. simpsoni was prevalent in many types of lowland country the higher incidence of human immunity to yellow fever along the forest margins was not likely to be dependent on larger populations of this species in such areas. Thus the results showed that in a thousand axils from open agricultural country the expected number of larvae of this species was 946, whereas in mixed country it was only 250 (26 per cent of the above) and in forest-edge country it was only 143 (15 per cent of the open country figure).

It now became important to establish that A. simpsoni was not breeding in other habitats in the mixed, partly-forested country or along the forest edge - in other words, to confirm that the above survey had in fact sampled the only important breeding-places. The following work was accordingly carried out: As the main breeding-place of A. simpsoni in West Africa is in tree-holes (v.s.), 500 of these were examined along the same routes. These yielded 2,145 larvae belonging to 21 species, but only 3 of these were A. simpsoni. Next 500 ground-pools were examined, and these yielded 1,143 larvae belonging to 16 species, but no A. simpsoni. Finally 500 pools in fallen leaves were examined, and once more no A. simpsoni were found, though 2,191 larvae belonging to 5 species were taken. A confirmatory survey of biting mosquitoes was then carried out along the same paths. In this work 1,046 mosquitoes belonging to nine species were collected, and of these 1,018 (or 97 per cent) were A. simpsoni. At the conclusion of this work the evidence was thus that in forest-edge as well as in other localities plant-axils form the only important breeding-place of A. simpsoni in Bwamba, and that it is probably less prevalent in forest-edge and mixed country than in open agricultural land with banana plantations.

Another study which was begun before the results of the first survey had been worked out and which, consequently, was also based on given numbers of entire plants, was a monthly survey of 100 colocasias (Xanthosoma) at Bundibugyo. This was continued for a year. Here, as in the case of A. gambiae at Kisumu, there seemed to be no correlation between the numbers of larvae and the rainfall (Haddow, 1942a). This is not surprising, as one shower is enough to fill the axils to capacity, after which additional rain cannot fill them further. Once full, they retain water for long periods. There was, on the other hand, a very close relation between the numbers of larvae and the cropping and growth of the plants. To give a single example, in April, 1942, there was heavy rain but few larvae, as planting was in progress and the axils of the new crop were still very small. Even in the succeeding month, in continuing heavy rain, the yield of larvae was poor, and this did not improve till June, when the plants had become large and the growth dense. For details of this survey reference should be made to the writer's paper (1948). In the course of the work 7,217 larvae were examined, of which 5,188 (or 72 per cent) were A. simpsoni. Ten larvae of A. aegypti were taken, and another species - Culex nebulosus - was added to the list. Breeding went on throughout the year as rainfall in Bwamba is well distributed, but there was one very short spell in early February, 1943, when the axils were apparently completely dried out. They refilled very soon, however, after a light shower, and the larval populations were re-established within ten days of this. Taking the overall yield from these colocasias for the entire year's work, it was found that the expected number of larvae per thousand axils was 1,169 and the expected number of A. simpsoni per thousand axils 840, a very high figure for a single species. After A. simpsoni the next commonest mosquito was U. ornata v.musarum, a harmless species.

While in Bwamba the axils might remain full virtually throughout the year it was obvious that in the drier parts of the Protectorate, with their more seasonal rainfall, this could not be the case. It therefore seemed worth while to find whether the eggs of A. simpsoni - already shown by Dunn (1926) to be drought-resistant - could be recovered from the dry debris at the foot of empty axils. This was done during the dry spell in late January and early February, 1943, which is mentioned above. The first collection, from 100 axils, contained some material which was slightly damp and it was most interesting to find that this still contained living larvae of mosquitoes and Psychodids. This first collection was accordingly discarded, and new material (scrapings) from 1,000 completely dry axils was obtained. When this was placed in water, larvae of A. simpsoni and Harpagomyia taeniarostris hatched out. The eggs of the second species, an interesting and highly specialised mosquito which does not suck blood, but probably, like other members of the genus, solicits regurgitated food from ants (Farquharson, 1918, quoted by Edwards, 1941), had not previously been recorded as drought-resistant. As a result of these observations it was concluded that, as in the West African tree-holes studied by Dunn (loc.cit.), A. simpsoni can weather dry conditions in Uganda, the eggs lying in the axils ready to hatch with the first rain. This must be of great importance in rather arid areas such as Karamoja District, where A. simpsoni occurs freely, though seasonally, in areas with little over 20 inches rainfall per year.

This work led to a consideration of conditions within the axil, temperature appearing to be the factor most likely to be of importance.

It was known that in Stegomyia spp. development may be slowed by low temperatures (Shannon and Putnam, 1934; Buxton and Hopkins, 1927). The latter workers had also found that the temperatures in colocasia axils in the area they studied were slightly lower than that of the surrounding air. In addition, Teesdale (1941) working on the Kenya Coast, had found that if he enclosed entire plants in mosquito-proof cages, A. simpsoni might continue to emerge for as much as 19 days thereafter, and this slow development again suggested cool conditions in the axils. It was decided that this matter should be investigated in Bwamba, and maximum and minimum thermometers were placed in a large Xanthosoma axil in the middle of a plot. Similar thermometers were placed in a Stevenson screen a few feet away. Readings were then taken for a week. The results showed that while the mean of the daily minimum readings in the axil was only about 0.5°C. below that for the screen, the mean maximum was 2.5°C. below that for the screen. The difference in mean range was 2.0°C. Thus the water in the axil was distinctly cooler than was the surrounding air and, further, its temperature was very equable, the absolute range being only 9.0°C. (from 17.0-26.0°C.). This point is not, perhaps, of great importance in a humid area with a very small daily temperature excursion, such as Bwamba, but it may be vital to larvae in arid districts where there is a very marked diurnal temperature rise, as in north Uganda.

The last of these minor investigations concerned the actual output of mosquitoes from a given plant, Xanthosoma once more being chosen for study. The point was raised by the difference between two thousand-axil samples from this plant, one taken at Bubukwanga and the other at Bundibugyo, about 5 miles away. The results were:

	Bubukwanga	Bundibugyo
Axils with water	663	418
Per cent potential foci occupied	67	63
A. simpsoni	615	1,321
Eretmapodites spp.	208	29
Other spp.	518	425
A. simpsoni per available axil	0.9	3.2

Thus, though there were fewer available axils at Bundibugyo, and though the percentage occupied was roughly the same in both areas, Bundibugyo yielded more than twice as many A. simpsoni larvae as did Bubukwanga. This was almost certainly due to the fact that the predatory larvae of Eretmapodites spp. are very prevalent at Bubukwanga, as is shown by the figures above. Each of these larvae dominates an axil, as mentioned elsewhere (Haddow, 1946) and it is known that the restless larvae of Stegomyia spp. are particularly prone to attack by predators (Shannon, 1931; Jackson, 1953). It is interesting to note that of the 518 larvae of other species taken at Bubukwanga, 504 were H. taeniarostris. The larvae of this mosquito spend much of their time simply lying at the bottom of the axil, with only short excursions to the surface and are thus more likely to escape a predator than are those of A. simpsoni. This comparison of samples raised the question of larval survival and of adult output as opposed to larval population. Teesdale (1941) had already tried to estimate output from plants in Kenya by caging them, but had had great difficulty in excluding predators such as spiders and mantids. A useful suggestion was, however, made by Mr. C. B. Symes during a visit to Bwamba and this was followed up. Symes suggested that one might gain a reasonable estimate of adult output by counting pupae, and in practice this method proved simple and useful. It may be mentioned that the pupal stage is very short and that pupae, being well armoured, are not readily killed by predatory larvae of other species. A small plot of colocasias was chosen and ten good plants were selected. All the axils were examined daily and the pupae were picked out, an equivalent volume of water being returned to the axils. The number of foci varied, as new leaves opened and old ones withered. The mean number of axils was 72, the mean number with water 53, and the mean number with larvae 43. Pupae were obtained on 29 days of the 30-day study. These belonged to the usual species. In all, 108 pupae were taken, of which 95 were A. simpsoni. The rate of output was thus very considerable. The implication was that an average plot of 500 plants might be producing almost 5,000 A. simpsoni per month.

Among the enemies of larvae in axils, which tend to cut down the output are firstly the predatory Eretmapodites spp. Then there are infections with Coelomyces africanus. This parasite had been studied in

Anophelines at Kisumu (Haddow, 1942a) where it was found that it may be present in adults as well as in larvae. In female mosquitoes it has the interesting property of destroying the ovaries. It must also be mentioned that where very heavy infestations of stalked peritrichous ciliates occur, metamorphosis is seldom completed, for reasons not understood at present. The axils are, moreover, the haunt of various species of tree-frogs, and these attack adult mosquitoes readily.

The main item in this whole programme of work on plant axils was a survey of larvae in 1,000 axils each of 12 different kinds of plant, and water measurements in 1,000 axils each of the same species, as it seemed likely that water-content might be one of the most important variables. It was not possible to carry out these two interlinked surveys simultaneously. Thus, in measuring water-content, a very finely pointed pipette must be used, while in collecting larvae a fairly broad-nosed one is best, and the axil is, in addition, washed out several times.

In making the water measurements and larva collections, it was necessary to avoid selection of axils, and thus if a plant were chosen the rule was that all axils must be examined and recorded. The work was all carried out in wet weather. Later it was felt that where water-measurements were concerned it might have been simpler to choose a plot of a given plant, fill all the axils to capacity, and then make the measurements. The method used was, however, more closely related to the actual state of affairs in nature.

The work was at first confined to the common plants discussed above, but later the survey was extended to include species such as the cannas. Finally, to make the study as complete as possible, plants from the rain-forest (Pandanus), from the highland agricultural area (Colocasia) and from the mountain forest (wild banana) were included. Had this not been done, results of considerable interest would have been missed, as will appear below.

The results of water measurement may be listed first, the mean axil content (cc.) being as follows:

Wild banana	166.9	Pineapple	4.2
C. esculentum	14.6	D. fragrans	3.5
P. chiliocarpus	9.3	Bitoke	3.1
X. sagittifolium	6.8	Menvu	2.9
D. steudneri	4.6	Canna spp.	2.8
Gonja	4.5	Sansevieria spp.	1.5

In the larva survey which followed one of the principal points emerging was the very close correspondence between the percentage of available axils colonised and the actual numbers of larvae taken. This matter, and others concerning the details of the survey have been discussed more fully in the writer's (1948) paper. For the present discussion the following data are sufficient:

Plant	Per cent available axils colonised	Total larvae taken	Total A. simpsoni larvae taken
Wild banana	82%	9,497	0
P. chiliocarpus	69%	1,816	0
X. sagittifolium	63%	1,775	1,321
Gonja	58%	1,221	1,203
Pineapple	49%	935	902
C. esculentum	24%	824	0
Sansevieria spp.	25%	218	218
D. fragrans	14%	150	126
Menvu	under 2%	32	32
Canna spp.	under 2%	10	10
D. steudneri	2%	8	8
Bitoke	under 1%	1	1

This table shows that with the exception of three plants a very large percentage of the larvae is always made up of A. simpsoni. These three will be discussed further below. From all further comment D. steudneri may be omitted, as the toxic water in the axils (v.s.) is obviously unsuitable for larvae. The few taken were all very small or else obviously sickly. It is doubtful if larvae can mature in the axils of this plant, as pupae were never found in it either in Bwamba or in other parts of East Africa surveyed subsequently.

With reference to the plants which did yield A. simpsoni, it is obvious that there is a relationship between the mean water content of the axils as revealed by the first survey and the numbers of larvae supported, as shown by the second. The relationship is not, however, a simple linear one where larval increase proceeds *pari passu* with increase in water content (though the correlation is quite high in the case of

A. simpsoni). If first the mean amount of water per axil for a given plant, as determined in the first survey, is multiplied by the number of water-containing axils recorded in the second, then a rough estimate of the total "expected" volume for the second survey is obtained. Using the figures thus obtained and the numbers of A. simpsoni taken it was found that the coefficient of correlation (r) is high ($r = +0.74$, and $t = 2.81$, where the value of t, at $P = 0.05$, is 2.45). Obviously, however, there must be a limit to this relationship as, given a large enough axil, its characteristics would approximate more closely to those of a small pool. Buxton and Hopkins (1927) found that certain specialised "small-container breeders" of the South Seas region preferred dishes of 15 cm.. diameter for oviposition when offered dishes 10, 15 and 30 cm. in diameter. These experiments, carried out with captive insects, now found a parallel in nature, as further analysis showed that a similar state of affairs occurred in Bwamba. Thus when plants with a very small capacity (mean axil content under 4 cc.) were grouped, it was found that there was only approximately 1 larva for each 12 cc. water. This group included Sansevieria, the cannas, menvu and D. fragrans. Where there was a medium capacity (mean axil content 4-8 cc.) the rate was strikingly different, approximately 1 larva for each 2 cc. water. In this group were included all the really important plants - Xanthosoma, gonja and pineapple. In the case of Pandanus and C. esculentum the mean content was high, falling within the range 8-16 cc. Here, however, though larvae were numerous, their actual concentration was reduced to 1 larva per 5 cc. water. Finally, in the wild banana with its enormous larval population and its huge axil content, the concentration of larvae was lowest of all, being only 1 larva per 13 cc. water. In this calculation, of course, larvae of all species have been included. Thus the most favourable plants, where competition for living space is concerned, were those with a medium axil content of 4-8 cc. It is perhaps a help in visualising this if the above figures are treated another way, to show the expected numbers of larvae per litre of water, as follows:

Group 1	Mean axil content under 4 cc.	83 larvae per litre
Group 2	Mean axil content 4-8 cc.	467 larvae per litre
Group 3	Mean axil content 8-16 cc.	210 larvae per litre
Group 4	Mean axil content over 16 cc.	78 larvae per litre

It is realised, of course, that here only a single variable is being considered, whereas in probability many come into play. To give only a single example it may be pointed out that Sansevieria, with a mean axil content of only 1.5 cc. yielded quite a large number of larvae, while bitoke, with a mean content of 3.1 cc. yielded only a single larva from an expected water volume of 347 cc. It does seem, however, in the light of this and of subsequent work, that the water-content of the axil is of considerable importance in appraising the importance of a given plant in relation to mosquito breeding.

The species list from this large survey remained much the same as before, there being only four additions. From Pandanus came the larvae of a new species of Uranotaenia, subsequently described as U. yovani (van Someren, 1951; Haddow, van Someren, Lumsden, Harper and Gillett, 1951). From the wild banana and from C. esculentum came larvae of Culex musarum. The wild banana also yielded a few larvae of two undescribed Culex spp., the final status of which has not yet been decided. A. aegypti occurred again, six larvae being taken in pineapple axils.

It was only when the survey of the commoner lowland plants had been completed that it was decided to include Pandanus. As mentioned above, the thickets formed by this plant are very difficult and unpleasant to penetrate. Accordingly, the sample came from the edge of the thicket, and this edge lay only about 50 yards within the main forest. There was an extensive banana plantation within five hundred yards, and between this and the forest margin there was much scrubby growth of the type through which A. simpsoni wanders freely in the neighbourhood of a breeding source (v.inf.). In spite of this the Pandanus did not yield a single A. simpsoni larva, though it was obviously a suitable focus for mosquito breeding, the thousand-axil sample yielding 1,816 larvae of other species. This was considered as a clear indication that, even where suitable plants occurred quite close to an extensive breeding-ground of A. simpsoni, they would not be utilised for oviposition if they grew within the limits of dense forest - even when very close to the edge. This observation was supported by the results of catches of adult mosquitoes in forest, which showed that while _A. simpsoni_ may enter the forest it does so in very small numbers and does not penetrate more than a few yards. The important corollary was of course that if yellow fever virus was active in the main forest, occurring in some animal host, as in South America, then _A. simpsoni_ could hardly be the vector in the animal-to-animal cycle.

At about the same time a few plants of _C. esculentum_ were found growing near Bundibugyo, and were recognised as very different from the usual lowland _Xanthosoma_. The axils were found to contain larvae, including _A. simpsoni_, and the plant was obviously a favourable one for mosquito breeding. Enquiries revealed that on the higher foothill slopes, at about 5,000-6,000 feet, below the Ruwenzori Forest, _C. esculentum_ replaced _Xanthosoma_ as a crop plant. A journey was at once made to study this situation, and a thousand-axil sample was taken, as part of the survey discussed above. Larvae were abundant, 824 being obtained. Not a single _A. simpsoni_ larva was found. It was replaced as a dominant species by _C. musarum_, of which 805 larvae were taken (98 per cent of the total sample). As the plant was known to be suitable as a breeding-place for _A. simpsoni_ in the lowlands, the matter obviously called for further study, the obvious conclusion being that _A. simpsoni_ was absent from these higher levels. A further sample from 1,000 axils was therefore collected at a point well north of the original area, and a third exactly similar sample at a point well to the south. These surveys which, like the first, were carried out in the altitude range 5,000-6,000 feet, confirmed the result. A sample was also taken from 1,000 axils of the wild banana, both below and within the Ruwenzori Forest. The result was again the same - among 9,497 larvae taken there was not one _A. simpsoni_. Once again _C. musarum_ was dominant, 9,235 larvae of this species being taken (97 per cent of the total sample).

There remained the possibility that at these higher levels _A. simpsoni_ might be breeding in tree-holes or some other habitat. To investigate this, 300 water-containing tree-holes were now examined, 100 each from three widely spaced localities in the Ruwenzori Forest. These yielded a typical montane fauna, including one species - Aëdes pulchrithorax - not previously recorded from Uganda, but no A. simpsoni. Finally, bored bamboos were studied. The East African mountain bamboo (A. alpina) is liable to attack by insects which bore small holes in the stems. In the bored sections a specialised fauna of mosquito larvae is to be found inhabiting the sugary liquid which collects at the foot of the sections. Three hundred such sections (100 each from three well-spaced localities) were examined, and yielded the typical fauna, including Uranotaenia garnhami, previously known only from the volcanoes of Lake Kivu, and Toxorhynchites ruwenzori (formerly Megarhinus ruwenzori van S.) known previously from a single specimen only. No A. simpsoni larvae were obtained. Catches of biting mosquitoes were also in progress, for other purposes, in the 5,000 - 7,500 foot zone and these also failed to yield A. simpsoni.

Work carried out by Mahaffy, Smithburn and Hughes in 1939-41, but not reported till much later (1946), had already shown that the main incidence of yellow fever was in the lowlands. Their figures showed that even when relatively unfavourable areas were included to permit the consideration of the lowlands as a whole, the overall incidence of immunity was about 12 per cent (in a sample of 1,720 sera) as opposed to only 4 per cent in the highlands (sample 334 sera). Many of the mountain Bakonjo people visit lowland markets weekly, and thus are exposed to lowland infections. Thus, with yellow fever prevalent in the lowlands, it was to be expected that some Bakonjo would acquire it. Subdivision of the sample into adults and children (i.e., individuals over and under 15 years respectively) showed, however, that the Bakonjo do not often acquire the infection early in life. Thus of 187 children from the highlands, not one was immune to yellow fever, whereas in the lowlands the overall rate was 5 per cent in a sample of 957. Thus it was concluded that only after many visits to the lowlands was a mountain Mukonjo likely to become infected with yellow fever. Later work carried out by Dick in 1946 gave an overall rate of 4 per cent for children under 4 years old from the North Spur, as opposed to 30 per cent from the similar age-group in the Lowlands (Dick, 1950), an even more striking result than that discussed above. While these facts were known, the reason remained obscure till the axil surveys had been carried out, when it became obvious that the key to the situation was the absence of A. simpsoni from the highlands. Using the new map it was possible to refer the numerous points from which bloods had been collected to the large-scale Uganda Government map of the North Spur of Ruwenzori, which has approximate contours. From this it became apparent that the critical level appeared to be about 5,000 feet, as in samples taken below this level the incidence of immunity was much higher than in those taken above. It was now considered important to find how this agreed with the upper distribution limit for A. simpsoni adults. While this work was not a part of the axil survey, it was so directly a consequence of the axil work that it is convenient to discuss the matter here.

A survey was made at various altitudes in the valley of the Tokwe River and along the flanking Bupomboli crest, a subsidiary mountain spur (Fig. 1). A. simpsoni was abundant in the lower valley, but did not occur above 5,000 feet, at which level only one was taken biting. At 5,500 feet the normal mountain mosquito fauna had been established. These actual heights are of local significance only, and must not be applied generally to East Africa. For example, A. simpsoni is known to occur at about 6,000 feet in parts of Kenya. Here, however, the habitat is sunny plains country, entirely unlike the cold, wet and stormy environment of the North Spur.

Thus the survey of Pandanus had shown that, if there were an animal host of yellow fever in the main forest, A. simpsoni was exceedingly unlikely to be the vector of the animal-to-animal cycle. On the other hand, where this species was absent, human infections were very scarce, and probably not locally contracted, from which it was concluded that there was probably no other important vector of the animal-to-man or man-to-man cycle in Bwamba, a view subsequently supported by the work of

Lumsden (1951a).

In concluding this account of the work on axils in Bwamba, it may be noted that 34,752 axils were examined, these yielding 26,002 larvae. The species list was, however, extremely limited, consisting of only 11 species and 1 group (as mentioned above, in the E. chrysogaster group females and larvae cannot be distinguished reliably). It is interesting to note that at a later stage, as described below, the writer made a wide survey in Uganda, covering 40 localities, well distributed over the Protectorate. One of the main objects was to map out the distribution of A. simpsoni. In this survey 29,424 axils were examined, but only one species (E. sylvestris v.conchobius) was added to the list, while five of the Bwamba species were not found at all. It may be added that this survey confirmed that A. simpsoni very rarely breeds in tree-holes in Uganda as, while the larvae were found in axils at every one of the stations (except in one mountain locality), and while it was often the only axil-breeding species found, not one specimen was taken in the concomitant tree-hole surveys, which included over 5,000 water-containing tree-holes from the same localities. The only localities so far found in Uganda where A. simpsoni may (rarely) be found breeding in tree-holes are Bwamba and Entebbe.

WORK ON MOSQUITOES OF THE GENUS ERETMAPODITES THEOBALD

Generally speaking, the author has avoided work on systematics and taxomy, regarding this as a field for the specialist, and most of the Bwamba material suspected to be undescribed or thought to be of special interest has been sent to Mrs. Ellinor C. van Someren, mosquito systematist in the Division of Insect-borne Diseases, Medical Department, Nairobi, Kenya. The collaboration with Mrs. van Someren, dating from 1942, has been a most helpful one. Many difficulties have been resolved by her, and on the other hand she has received from Bwamba material for the description of 14 new species and subspecies, namely, 1 Megarhinus, 1 Uranotaenia, 1 Ficalbia, 4 Aëdes, 4 Eretmapodites and 3 Culex spp. The other new forms from Bwamba have been described by other workers.

Only in the case of the genus Eretmapodites has the writer done much systematic work, and the studies on these highly specialised mosquitoes will now be discussed briefly.

When work was begun in Bwamba it was already known that the period of active virus circulation in mammals infected with yellow fever virus was very short, as shown by the work of Bauer and Mahaffy (1930) and various other papers. It was thus obvious that in any animal-to-animal cycle, while the animal concerned was a host, it could not be considered a reservoir, the real reservoir of infection being the arthropod host. This point has been discussed more fully by Bugher, Boshell-Manrique, Roca-Garcia and Osorno-Mesa (1944), but it is really implicit even in the earliest work on the mosquito vectors of yellow fever, as it was soon found that infection in the mosquito persists for life (Reed, 1901). Bauer (1928) had shown that certain mosquitoes other than A. aegypti could transmit the virus by bite under laboratory conditions, and the list of potential vectors was lengthened by various workers who followed his lead. Among the mosquitoes tested by Bauer was Eretmapodites chrysogaster (though in the light of present knowledge his material would be referred merely to the E. chrysogaster group, as more precise determinations can be made only by examining the male terminalia). An interesting point brought out by Bauer was that in infections produced in monkeys by the bites of E. chrysogaster the incubation period might be prolonged. This was a point of some importance, any process tending to lengthen the period of virus activity in the mammalian host being regarded as helping the persistence of the virus, and it was this finding which aroused special interest in the genus.

It was obvious that in Bwamba yellow fever was associated with the main forest. As A. simpsoni did not enter the forest to any significant extent, it seemed likely that the man-to-man chain of infection was most likely to be initiated by something coming from the forest, and at first a mosquito was considered to be the most probable link between the forest and the African population outside. The first work in banana plantations revealed that Eretmapodites spp. (previously regarded as essentially forest mosquitoes) were abundant in this habitat, the larvae being dominant in leaf-pools, common in axils and occasionally present in domestic receptacles around huts. Work in the forest showed that this genus was equally common there, with breeding-places in leaf-pools, tree-holes and empty snail shells (particularly the large shells of Achatina and Limnobius spp.). It seemed quite likely that these mosquitoes, all of which bite man viciously by day, might be the postulated link between the forested and agricultural areas, and for some time they were studied rather intensively, more particularly in view of the fact that one species was known to be a vector under laboratory conditions.

Bauer had noted that there was some predatory activity among his larvae, and J. O. Harper (private communication) had also found some predatory activity in a Bwamba species. The habit, however, received little attention till the writer found that it was common to all the species studied, and that while it was possible to rear out larvae without providing prey, all were keen predators and matured much more rapidly when fed on mosquito larvae. All possessed a structural modification which had not previously been described, namely, a bunch of stiff hairs in the mouth-brushes, these hairs having comb-like terminations similar to those found in the predatory larva of Culex tigripes, and obviously very suitable for holding struggling prey. Experiments on predatory activity had been carried out previously at Kisumu, with C. tigripes as predator and A. gambiae as prey (Haddow, 1942b). Similar work was now undertaken with the larvae of various Eretmapodites spp., the prey being A. simpsoni larvae. It was found that large larvae of E. chrysogaster devoured a mean of 6 A. simpsoni larvae each in 24 hours. Those of E. semisimplicipes devoured a mean of 8, E. inornatus 2, E. penicillatus 5, E. ferox 9 and E. leucopus spp. productus under 2. The larvae of E. oedipodius spp. parvipluma, which also occurs in Bwamba, were not tested at that time, but subsequent experience at Entebbe has shown that they also are predatory. Other experiments showed that pupae are also attacked but usually escape, probably on account of the hard integument and the violence of their swimming movements when disturbed. Yet others showed that E. chrysogaster larvae will attack their own kind, large or small, but seldom with success, once more tough integument and powerful swimming movements aiding escape. The larva of E. ferox is the most active predator of all, and it is interesting to note that when two larvae of this species are found together in an axil in nature, they may be seen to attack each other repeatedly, and examination will show that most of the setae have been cropped off and that usually the anal gills have been much damaged. Larvae of E. ferox do not lie in wait for prey like most predatory mosquito larvae, but move about actively in search of food. When a larva is caught it is shaken and worried in a most vicious manner and completely consumed in about ten minutes.

Eretmapodites is the only mosquito genus confined to the Ethiopian and Malagasy zoogeographical regions. It is, however, closely allied to the Oriental genus Armigeres Theo., not only in appearance, but also in biting-habits and in larval and adult habitat. When work was begun in Bwamba the genus was very poorly known and museum material was quite inadequate, many of the immature stages being unknown, and the adults represented by a few specimens only. The examination of large samples of adult mosquitoes soon showed that the distinguishing characters given by Edwards (1941) were unreliable in many cases, particularly in the common E. chrysogaster group. A good deal of collection and breeding-out was therefore undertaken, and this resulted in the first descriptions of the larva and pupa of E. inornatus, the larva, pupa and adult female of E. penicillatus (with the discovery of a character for distinguishing the females of these two species), and the larva and pupa of E. leucopus spp. productus. A considerable amount of information was also gathered on habits and habitat, E. chrysogaster was colonised in the laboratory and its curious copulatory behaviour noted.

With reference to the form referred to in this paper as E. ferox, a more detailed note is necessary. Edwards (1916) described as E. dracaenae a species from Sierra Leone and the Gold Coast. Subsequently the same species was recorded from Central Uganda by Hopkins (as quoted by Edwards, 1941), and it was found in Bwamba by Harper (private communication) and Gibbins (1942). When, however, the present writer began to examine series in Bwamba, examination of the male terminalia revealed many differences from the figures given by Edwards (1916 and 1941). It may be mentioned that Edwards' descriptions were brief and incomplete. On this basis it was decided that the Bwamba material should be assigned to a new species, E. ferox, and all stages were described. Examination of the terminalia of the type of E. dracaenae in the British Museum showed that some of the characters thought to be characteristic of E. ferox did, in fact, occur in E. dracaenae, Edwards' figures, like his text, being very incomplete. As various differences still held good, it was considered that E. ferox was a good species and there the matter rested till 1956. In that year Dr. B. O. L. Duke sent to the writer the terminalia of an Eretmapodites male of the dracaenae-ferox group from the Cameroons, i.e., from the normal range of E. dracaenae. The terminalia were, however, identical to those of the Bwamba specimens. The writer therefore suggested reopening the question of the validity of E. ferox, and Duke's material was sent to Mr. P. F. Mattingly, at the British Museum. As it is known that, in balsam mounts, structures in mosquito larvae and male terminalia may in time become excessively transparent, Mattingly now dissolved out the type terminalia of E. dracaenae from Edwards' balsam mount and remounted in a gum-chloral medium. Various structures now became apparent, and it was obvious that Edwards' material was identical to Duke's and also to the Bwamba specimens. In the near future, therefore, E. ferox will become a synonym of E. dracaenae. As, however, this point arose after the draft of the present communication had been completed, it is convenient to retain the name E. ferox here.

The systematic work in Bwamba showed that Edwards' (loc.cit.) keys were no longer adequate and they were, in addition, unreliable in some parts. New keys to the larvae, pupae, males and females were therefore prepared. For fuller details of this work, reference should be made to the original paper (Haddow, 1946).

It may be mentioned that at a later date much material was collected in Bwamba for Mrs. van Someren, who paid several visits to the field station. This resulted in the addition of three known species to the Bwamba list, and also to the discovery of four new species of the E. chrysogaster group (van Someren, 1949).

Yellow fever virus has not, up to the time of writing, been isolated from mosquitoes of this genus. In 1944, however, three strains of Rift Valley fever virus were isolated from Eretmapodites spp. caught in the Semliki Forest (Smithburn, Haddow and Gillett, 1948). In addition to this, Semliki Forest virus has been isolated from E. grahami in West Africa (Macnamara, 1953). This virus was originally isolated from mosquitoes of the Aëdes abnormalis group caught in Bwamba (Smithburn and Haddow, 1944). Thus, though probably not of importance in relation to yellow fever, Eretmapodites spp. have proved to be of interest in connection with two other viruses. Further, in the case of the very important virus of Rift Valley fever, it has been shown that E. chrysogaster is a good laboratory vector (Smithburn, Haddow and Lumsden, 1949a).

THE MAIN PROGRAMME IN BWAMBA

Work carried out during 1942

The main programme in Bwamba centred on catches of adult mosquitoes and the observation and collection of monkeys and other animals. The mosquito side of the work soon became divided into intensive catches made in the search for yellow fever and other viruses, and investigative catches concerned mainly with the study of biting-habits, habitat and distribution.

The method employed in intensive routine work was to collect and accumulate adult mosquitoes for five days, after which they were sent alive to Entebbe by car. On arrival the survivors - usually a very substantial percentage of the total catch - were ground up in diluent, after which the suspension was centrifuged and the supernatant fluid drawn off for inoculation into mice and rhesus monkeys. During the first year mosquitoes found dead on arrival at Entebbe were discarded. Alternate weeks were given up to these routine catches and to investigative catches.

At first very large stationary baits were used - often more than 100 people, standing in groups, while a small number of trained boys collected the mosquitoes which came to bite. It was soon found, however, that a few skilled moving catchers would produce far more mosquitoes than would large stationary groups. Here, of course, another factor, movement and disturbance of the undergrowth, has an effect. The skilled catcher moves along for a few yards, gently shaking the leaves and twigs, and then stands still waiting for a few minutes before moving on again. This is a highly effective method. By the end of the year a team of about twenty catchers, using this method, carried out all the routine work. They moved actively through plantations and forest, collecting the mosquitoes which came to bite and also collecting separately those which were found resting on the undergrowth. By the end of the year this team, which started by making catches of 50-150 mosquitoes per day, was occasionally taking as many as 1,000 specimens in a single morning. It will be remembered that at this time there was very little information about the biting-habits of forest Culicines, and from these routine catches a good deal of information gradually accumulated concerning the relative preference of different species for different environments, and also about which species did and which did not bite man. Collections of biting mosquitoes were almost always made up mainly of _Taeniorhynchus_, _Aëdes_ and _Eretmapodites_ spp., with abundant _Anopheles_ spp. in some areas. Collections of mosquitoes from the undergrowth almost always yielded more mosquitoes than were taken biting, and in such collections the genus _Culex_ was almost always by far the best represented. The relatively small numbers of _Uranotaenia_, _Ficalbia_ and _Theobaldia_ spp. also came mainly from the undergrowth. An interesting point was that a fairly large percentage of the resting _Culex_ was usually made up of fed specimens. Unfortunately, the delicate methods later developed by Weitz (1952) for the identification of the hosts from which such meals have been taken were not then available.

While work in plantations remained the main concern, the number of species encountered was rarely large, and sorting was simple. When work began in the forest, however, a bewildering variety of different species was encountered and the matter was further complicated by the presence of various undescribed forms and by the fact that many of the others had previously been recorded from West Africa only. The work of sorting was thus very slow at first, more particularly as the mosquitoes had to be identified while still alive. But for the fortunate chance that Edwards' book on the Culicines of the Ethiopian Region had just appeared (1941), the identification of most of the mosquitoes encountered in the forest could not have been undertaken at all.

At an early stage it was realised that the range of individual variation in most species was much greater than was indicated by the available descriptions. Throughout the work in Bwamba, therefore, a point was made of collecting long series for taxonomic study at Nairobi by Mrs. van Someren, whose contribution will be discussed further below. In a few cases such studies were carried out in Bwamba. Thus when it was found that almost every supposed diagnostic character of A. metallicus (with the exception of the scaling of the pre-scutellar area) might also be found in A. simpsoni, a good deal of time had to be spent in checking the whole question of the distinction between the adults of these species.

The collecting and sorting procedure was designed to permit the fastest possible disposal of a large and varied catch. Each mosquito was caught in an individual tube which was then lightly plugged with cotton wool. The tubes were fairly short, the most usual sizes being 15 x 75 or 15 x 80 mm. They were round-bottomed and were made of "Pyrex", "Monax", or other hard glass, to stand up to the wear and tear of field work. Sorting was carried out with a low-power hand-lens (6x or 7x) as it was found that for rapid work a large, brilliant field and good depth of focus were essential. If a magnification over 7x is required then it is likely that in any case a low-power binocular microscope will be necessary. After the preliminary sorting into wire test-tube racks, the identified mosquitoes were released into Barraud travelling cages which, if space is short, will hold as many as 400 - 500 mosquitoes each, though it is preferable not to exceed 200 - 300. Banana was the regular food, and humidity was maintained in the cages by draping them with damp cotton cloth.

Even at an early stage a major difficulty was met. As the number of species was considerable (the final Bwamba list included 160 forms) it was not possible to allocate a separate monkey and mouse groups to each. Thus, while a few suspect species such as A. simpsoni were selected for individual treatment (as were also some of the particularly abundant species) generic or subgeneric groupings had to be employed in most cases. For this reason it sometimes happened that a strain of virus was isolated from a group containing numerous species, and it was not possible to tell from which of these it had come. Nowadays, with better facilities available, each group is ground up separately and, though the suspensions may be pooled for the primary inoculations in order to economise laboratory animals, an aliquot of each is retained separately, deep-frozen. Should virus be isolated, the various suspensions can be reinoculated separately, to find from which the agent was derived. At the time concerned deep-refrigeration apparatus was not available in East Africa.

The first catches of 1942 were made in banana plantations, with the object of finding whether yellow fever virus could still be obtained from A. simpsoni. The work was confined to the study area described above, and catches were carried out in five localities. By the time that one catch had been made in each, 2,276 mosquitoes had been taken. As the catches were all made in or around plantations, the percentage of A. simpsoni was very high, 2,207 (or 97 per cent of the total yield) belonging to this species. Lot No. 5 came from Bundinyama and of this particular collection 395 A. simpsoni were inoculated into mice and a rhesus monkey at Entebbe. A fatal yellow fever infection followed in the monkey and the virus was successfully isolated. This was a result of major importance, as it was considered as presenting conclusive evidence that in Africa, as in South America, there must be an animal-to-animal cycle, independent of the infection in man. Thus the entire human population of Bwamba had been vaccinated against yellow fever about 11 months previously and the infected A. simpsoni must therefore have picked up the virus from some other host. Further, as A. simpsoni was by this time known to be very scarce in the main forest, it seemed likely that the postulated animal host must be of a group common to forest and plantations or, more probably, of a group which left the forest from time to time to feed in plantations, the main habitat of A. simpsoni. This was true of certain monkeys, and monkeys were already known to be important hosts of the virus in South America. This virus isolation has been reported in detail by Smithburn and Haddow (1946).

During the incubation period of the disease in the monkey inoculated with Lot 5, work had gone on in the plantations, and various further mosquito lots had been collected, sent to Entebbe, and inoculated into animals, but immediately the virus was isolated and identified, work was switched to the forest and the forest mosquitoes. As it was possible to delimit accurately the area from which the virus had been isolated (a single plantation) the nearest forest area was the obvious choice for intensive catching. It so happened that this was actually contiguous to the plantation. The latter was close to the steep wall of the Manjuguja Valley, the floor of which was covered by a dense strip of relict forest, extending from the main Semliki Forest. Below the plantation a subsidiary strip of dense high-canopy growth branched off from this valley forest and extended up the valley wall to end almost at the edge of the banana area. The first forest catch was made here, but virus was not obtained. As it was felt that the zone of virus activity might well have moved further up the Manjuguja or Tokwe valleys, two catches were made at Kanyamwerima, where this particular system of relict forest strips ends. Virus was not obtained there, and work at Bundinyama was taken up again. The 16th large routine catch of the year was made there in August, yielding 3,151 mosquitoes, belonging to 21 species and groups. Among these was a small lot of the Aëdes abnormalis group. The species of this group can be distinguished reliably only by examination of the male terminalia; in the case of females, more particularly when they must be sent to headquarters alive, a "group" determination is all that can be given with confidence. This is particularly true in Bwamba, where the final list included eight members of the group, three of them previously undescribed. From this small lot of mosquitoes, of which 130 were inoculated into a monkey and mice, a hitherto unknown agent, later named Semliki Forest virus, was isolated. A note on this agent will be found in Appendix 2.

The isolation of Semliki Forest virus gave considerable impetus to the work as, apart from its intrinsic importance, it showed that the methods previously used successfully in the case of plantations and A. simpsoni would also be useful in the case of forest and forest mosquitoes. Efforts to isolate virus were now redoubled, but no further isolations were made in 1942, though by the end of the year 25,038 mosquitoes had been taken in routine catches, of which 19,754 had been sent to Entebbe for inoculation. This may not seem a very large number, but it is to be remembered that the writer had only one experienced African assistant. All other staff - mainly illiterate - had to be trained, the building of the camp had to be seen to, a map made, and transport arranged for mosquitoes, animals, food, etc., over a difficult mountain road.

Another very necessary but time-consuming section of the routine work was the collection and identification of wild animals, of which 431 (a large proportion were rodents) were sent to Entebbe during the year for yellow fever laboratory studies. The results of the investigations on these animals and on others collected in subsequent years have been reported by Smithburn and Haddow (1949a, 1949b). Briefly, this work showed that, of the groups tested, only the primates were really susceptible to yellow fever, though some of the others did circulate small amounts of virus with the subsequent development of immunity. Further, every species of monkey and lemuroid tested was found to permit multiplication of yellow fever virus and its free circulation in the peripheral blood with the subsequent appearance of specific antibodies.

It became apparent, before the end of the year, that in the Semliki Valley the trapping of monkeys would always be difficult. Thus in the evergreen rain-forest food is abundant all year round, various species rarely leave the high canopy, and some of the more interesting monkeys are unlikely to be attracted by the ordinary trap baits. For example, the diet of Colobus spp. consists almost entirely of leaves and green shoots. On the other hand, it was also apparent that the monkey fauna was an unusually rich one. The final list comprised 12 species (not counting the lemuroids), including two not previously recorded in East Africa, while a further three, all quite common in Bwamba, had previously been considered as rarities in Uganda. It was also obvious that the population was very high, particularly in forest-edge and swamp-forest localities. It was therefore decided to augment the trapping programme by shooting monkeys and collecting blood sera for test. Towards the end of the year this was begun and, when monkeys sent alive are also included, 13 Bwamba monkeys were successfully tested during 1942. Seven of these showed immune bodies to yellow fever virus, and this was taken as implying that monkeys were probably to be considered important mammalian hosts of yellow fever in this part of Africa as in South America. It should be mentioned that before the end of the year tests were carried out on the sera of 37 other Bwamba animals of various groups, no positives being found. From an early stage very detailed notes and measurements were kept on each monkey shot, with records of environment, exact locality, etc. (Haddow, Smithburn, Mahaffy and Bugher, 1947; Haddow, Dick, Lumsden and Smithburn, 1951; Haddow, 1952a). A considerable mass of information on monkey natural history was thus gradually accumulated, and this was augmented by long periods spent in observing monkey bands. From this it soon became apparent that very little was known of the behaviour of the local species. For example, it had not been realised how strictly arboreal were the Colobus and Cercocebus spp. A point of great importance which was soon firmly established concerned the range of daily movement. It had been considered previously that monkeys were rather far-ranging creatures and, in species living in fairly open woodland country, this has a certain degree of truth. Work in Bwamba soon showed, however, that in the case of forest monkeys, once individual bands were known well enough to be recognised, they would be found every day in the same small territory, never ranging over more than a square mile, and generally over only about half of this area. The territorial boundaries of bands could often be recognised, and might consist of the gap in the canopy along a forest stream, the main road, or some similar feature. The obvious and important implication was that a monkey found to be immune to yellow fever - or to some other virus infection - would in all probability have experienced the infection within the small area where it was shot. Activity of the virus could now be pinpointed to within, roughly, one square mile, and thus a new line of study could be undertaken, the plotting of zones and areas of virus activity in the main forest. In the succeeding years this part of the work steadily increased in importance.

Investigative mosquito work in 1942 was at first largely concerned with the behaviour of A. simpsoni. The first study undertaken concerned the distribution of biting females in a large plantation. Comparable units worked simultaneously in a thin, newly-planted area, a dense jungly part of the plantation, much overgrown by vines and creepers, a small village clearing (about 40 yards across), the plantation edge and the main road which cuts through the plantation. The results showed a most definite "edge effect" and have since been quoted by Audy (in press) as an example of that effect. A. simpsoni is, in fact, a mosquito of the plantation edges and of what Audy has so aptly termed "parang vegetation", created by wasteful shifting cultivation. The numbers of A. simpsoni taken were as follows (other species made up only 4 per cent of the total catch):

Road	3 (1%)
Village clearing	7 (3%)
Dense growth	11 (4%)
Sparse growth	75 (29%)
Plantation edge	165 (63%)

It is interesting to record that the best station, the plantation edge, was divided from the poorest, the road, by only 20 yards of grass cut to about ankle height.

The next catches were designed to investigate the preference of A. simpsoni for different kinds of bait of roughly equivalent bulk. The baits were a small African child, 2 small baboons, 2 kids and 6 fowls, all placed side by side. Catching was carried out simultaneously on all. The results showed that there was a very definite preference for human blood, the numbers of A. simpsoni taken being as follows:

Man	58 (61%)
Fowls	23 (24%)
Goats	12 (13%)
Baboons	2 (2%)

Finally, Gibbins (1942) had noted that A. simpsoni seemed to prefer to bite the head. The writer's field observations were in agreement, but it was felt that perhaps this merely indicated a preference for a zone of activity a few feet above ground level, and that a more critical trial was required. A group of naked human baits was used. One pair sat, one pair stood, and one pair lay down. They changed position every ten minutes to eliminate the effects of individual attractiveness. In all 198 A. simpsoni were taken. When the total results were considered, regardless of posture, it was found that 99 (50 per cent) were taken on the head alone. If the count was extended downward to include the shoulders and upper chest to the nipple line then the number taken was 140 (71 per cent of the total catch). When the various postures were considered separately, this effect was still quite obvious, as shown below:

Posture	Catch on head alone	Catch on whole area above the nipple line
Standing	44%	68%
Sitting	62%	85%
Lying	42%	53%

The sitting posture leads to a rather complicated disposal of the limbs, and in later work standing and lying positions were the only ones studied. The body was divided into four portions: (1) The whole region above the nipple line. (2) The region between the nipple line and the trochanteric line (i.e., a line drawn through the great trochanters of the femora). (3) The region between the trochanteric line and the knees. (4) The region below the knees. When the figures of the above catch are reassembled to fit this scheme, the results are as follows:

Position	Standing	Lying
Above nipple line	57 (68%)	24 (53%)
Nipple line - trochanteric line	17 (20%)	10 (22%)
Trochanteric line - knee	4 (5%)	7 (16%)
Below knee	6 (7%)	4 (9%)

The reasons for this biting-distribution remain obscure. It was thought at first that the blackness of African hair might be the reason (A. simpsoni alights very readily on such objects as a black hat). As carbon dioxide seems to attract certain mosquitoes it was also felt that there might be more carbon dioxide output from the hair area, though later it was realised that the carbon dioxide output in the breath must be very much greater. The question about hair was, however, settled in a later experiment. Many of the Baburaburi (Baamba) are very light in colour, but their hair is black. A boy with light coffee-coloured skin who had just shaved his head was chosen, and a catch made (he stood and lay for alternate 5-minute periods) with the following results:

Position	Standing	Lying
Above nipple line	83 (62%)	40 (39%)
Nipple line - trochanteric line	33 (25%)	29 (28%)
Trochanteric line - knee	12 (19%)	15 (15%)
Below knee	5 (4%)	19 (18%)

Thus, even when the head was shaved, the upper part of the body remained the most attractive, though possibly less so than in the first experiment.

Some unpublished work of the same nature, using a single bait as in the second experiment, has shown that the behaviour of certain other mosquitoes differs entirely in this respect from that of A. simpsoni. Thus, while in the four species shown below the lower legs and feet were the part most frequently bitten in standing baits, the distribution changed entirely when the bait lay down. The general conclusion was that these species, unlike A. simpsoni, showed no preference for any particular part of the body, but were merely flying and biting just above ground level. The results were as follows:

Taeniorhynchus fuscopennatus

Position	Standing	Lying
Above nipple line	24 (18%)	50 (24%)
Nipple line - trochanteric line	30 (22%)	83 (39%)
Trochanteric line - knee	33 (24%)	42 (20%)
Below knee	49 (36%)	35 (17%)

Taeniorhynchus uniformis

Position	Standing	Lying
Above nipple line	15 (13%)	25 (23%)
Nipple line - trochanteric line	32 (27%)	45 (41%)
Trochanteric line - knee	20 (17%)	18 (17%)
Below knee	51 (43%)	20 (19%)

Eretmapodites chrysogaster gp.

Position	Standing	Lying
Above nipple line	0 (0%)	17 (15%)
Nipple line - trochanteric line	0 (0%)	49 (45%)
Trochanteric line - knee	8 (2%)	36 (33%)
Below knee	327 (98%)	8 (7%)

Anopheles gambiae

Position	Standing	Lying
Above nipple line	3 (1%)	125 (43%)
Nipple line - trochanteric line	18 (9%)	60 (20%)
Trochanteric line - knee	15 (7%)	39 (13%)
Below knee	172 (83%)	69 (24%)

More work is necessary to elucidate these results, but it has not yet been carried out, though recently Muirhead Thomson (1951b, 1954) has produced some interesting figures on an allied subject. His conclusions are that different age-groups are not equally attractive to Anopheline mosquitoes, adults being bitten more freely than children. The attack of mosquitoes on a given individual may thus be governed by numerous factors. Time, microclimate, flight level, physiological state, what we call for convenience "individual attractiveness" and even in some cases, particular body areas may all enter the picture. It is felt that the whole subject of the preferences of mosquitoes for certain individuals and in some cases for particular levels or parts of the body is one that calls for further attention.

Turning now to work carried out in the forest during 1942, it was first established that for detailed work hand-catching was preferable to catching with trap-nets. Thus, if mosquitoes entering a trap-net of the Shannon type (Shannon, 1939) were not caught continuously, many were lost. These tests were carried out with monkeys of several species, fowls and humans as bait. Thus in a test with fowls the only mosquitoes attracted were Aëdes cumminsi. During the first two hours mosquitoes were collected as they entered the trap, and 24 were taken. During a second similar period they were counted as they entered, but were collected at clock hours only. In two hours 21 entered, but of these 15 escaped. A careful catch with human baits gave a similar result. One bait was inside the net, another outside. One day catch and one night catch were made (2 hours each). Half-way through each catch the baits changed places. Catching on the exposed bait was continuous. In all 113 mosquitoes alighted, 107 being taken. Thus only 6 escaped. Catching in the net was at clock hours only, but all mosquitoes entering were counted. Only 50 entered and of these 35 were caught and 15 escaped. Thus far fewer were attracted, and more escaped. A trap-net has only one advantage - the fact that it is supposed to work without the necessity for a lamp which may influence the results in night catches. As, however, it is obviously necessary (unless many mosquitoes are to be lost) to catch all the time in the net, and so to use a lamp continuously, this advantage is theoretical rather than actual. Some other types of trap, such as the portable stable trap (Magoon, 1935) are excellent for certain purposes, but were far too cumbersome to be considered for the purpose in mind. As a result of this and other work the writer has used hand catching in all his subsequent work on mosquito biting habits and, where night catches were concerned, has merely used the dimmest light which will permit the mosquitoes to be seen. That the use of such dim light does not introduce a serious fallacy is shown by the fact that diurnal species stop biting at sunset whether a dim lamp is used or not.

The results of routine catching in forest had produced some very unexpected results because, as has been mentioned above, virtually nothing was known at that time of the biting habits of African forest Culicines.

In the majority of species it was not even known whether man was attacked, much less at what time biting was most active. Both in forest and plantations the results of large routine catches revealed the presence of a surprisingly large and varied group of species which bit mainly or entirely by day. Others preferred to bite at night and a few appeared indiscriminately in both periods. It was obvious that study of the biting habits was necessary as, for example, a mosquito which bites mainly by night in the forest or in the open is unlikely to be a good vector of disease from man to man though it might be a good vector from animal to animal. The obvious diversity of habits and of times of main activity led to the revival of the idea developed at Kisumu for an investigation covering the whole 24 hours, with the catch divided into one-hour groups. This work was begun late in the year and only five such catches had been completed by the end of 1942. At first they were carried out with fairly large human baits and skilled collectors who caught the mosquitoes coming in to bite. By far the greatest difficulty was the fear of the African staff for the forest by night, not so much on account of the prevalence of large game animals such as elephant and buffalo, as of a deep-seated dread of the supernatural. It took a good deal of time and trouble to overcome this feeling and, before beginning 24-hour catches, a series of small night catches of slowly increasing length had to be undertaken to accustom the staff to being out-of-doors after dark. The results of these first 24-hour catches were very instructive, but they need not be discussed here. They will be considered below with the rest of the series concerned.

The first of these catches were carried out in various forest localities, some of which were outside the original study area. Two journeys were also made through the main forest to the Semliki River, short investigative catches in forest and in tents being made in both cases. The fringing forest of the Semliki River proved to be a very fine collecting ground, both for mosquitoes and for animals, and much work was done there later. The very first journey, however, revealed the most interesting point of all, namely, that *A. gambiae* was exceedingly common along the banks and could be taken biting in substantial numbers both in tents and outside. A further point of interest was that quite a large number could be taken biting in forest by day, though this species is usually nocturnal. As A. gambiae is among the most endophilic and anthropophilic of African mosquitoes, its presence in forest entirely uninhabited by man, at a distance of six to eight miles in a straight line from the nearest huts was a cause for considerable surprise. Subsequently it was shown to be abundant in various other uninhabited forest localities, particularly at Mongiro and Mamirimiri, areas which will be discussed more fully below. A point of great interest was that where huts were present in any number along the forest edge A. gambiae at once assumed its usual habit of strict endophily and could not be taken biting in the open. The work on A. gambiae has been discussed more fully elsewhere (Haddow, 1945a, 1945b; Haddow, Gillett and Highton, 1947).

The other finding of major interest was the comparative prevalence of A. aegypti larvae in tree-holes. During the surveys along roads and footpaths referred to above in the section on plant-axils, 500 tree-holes were examined. Of these 422 were found to contain larvae, the total catch being 2,145 larvae of all species. Of these 132 (6 per cent) were A. aegypti. This was in agreement with the unpublished records of Harper, and further correspondence with his early results came from the observation that the larvae of this - usually - most domestic of all mosquitoes, were not particularly common in receptacles in and around huts, the classic breeding-place. Adults, moreover, could not be found in huts, though a few were taken in forest. The conclusion was reached, therefore, that in Bwamba two of the most important domestic mosquitoes of Africa, A. gambiae and A. aegypti, had been found in what might be described as the wild condition.

These findings, while of exceptional interest, were not unprecedented. Thus Wiseman, Symes, McMahon and Teesdale (1939) had found extensive outdoor breeding by A. aegypti at Mombasa, larvae being found in a great variety of small containers, including tree-holes and plant axils. An 80-day search of outdoor resting places had yielded a great number of adults, 203 males and 585 females being taken in resting places of the most varied nature. In West Africa Dunn (1926; 1927a, b and c; 1928) had found A. aegypti eggs and larvae in tree-holes and in other outdoor breeding places, sometimes as much as 500 yards from the nearest house, and there are various other records of this type. The Bwamba observations, however, probably represent the first discovery of breeding-activity by this species in completely uninhabited forest with no houses anywhere near. Similar results were reported later by Garnham, Harper and Highton (1946) from the Kaimosi Forest in Kenya. The scarcity of adults in native huts had also a precedent in the work of Hopkins at Kampala, Uganda (as quoted by Edwards, 1941).

In the case of Anopheles gambiae there were also prior records of this species from uninhabited localities. Thus Symes (1930) had found it abundant at the Little Lume River near Taveta, Kenya, and he had concluded (1931) that it subsists on animal blood till human settlement brings about an increase in numbers and a change of habits. Similarly G. H. E. Hopkins (private communication) had found it abundant in an uninhabited area on the Aswa River, Uganda. With reference to outdoor biting this had already been recorded by Kerr (1933) in West Africa and Wilson (1936) in Tanganyika. The observations in Bwamba were the first, however, to reveal a huge population of this species living and biting entirely in the open, in an extensive forest area uninhabited by man. These findings were, in fact, received with some scepticism at first. They aroused a good deal of interest, however, and their correctness was confirmed by visits over a period of years from various well-known workers on mosquitoes, namely, P. A. Buxton, G. H. E. Hopkins, C. B. Symes, P. C. C. Garnham, J. O. Harper, E. C. C. van Someren, R. C. Muirhead Thomson, and F. L. Soper. The last-named visited Bwamba during a rather dry spell, when it might have been thought that few A. gambiae would be found. He gave one catching-tube each to 12 collecting boys and sent them into a dense forest patch with instructions to catch one resting A. gambiae each and to bring it back. The last boy had returned in less than five minutes. This result may give some idea of the overwhelming prevalence of this mosquito in certain uninhabited forest areas in Bwamba. To give a further example, it may be mentioned that in the uninhabited forest along the Semliki River, most of which lies 6-7 miles from the nearest huts, 16,430 mosquitoes were collected during the next few years. Of these, 2,687 (or 16 per cent) were A. gambiae. Finally the case of the dense swamp forest at Mongiro may be mentioned. Much work was done in this area, and between 1942 and 1948 the number of mosquitoes taken there in routine and investigative catches was 74,783. Of these 37,810 (or 51 per cent) were A. gambiae. At that time there were no huts anywhere near Mongiro. These figures are in striking contrast to results recently published by Gillies (1955) who, working in a very malarious area in Tanganyika, could recover only 14-15 female A. gambiae per acre from outdoor resting places, after the most diligent search.

As it was already known that A. gambiae cannot transmit yellow fever virus (Philip, 1930), it might have seemed that this species was of no importance in relation to the enquiry. Large numbers were, however, collected and inoculated during the Bwamba work, as Philip (loc.cit.) had shown that it can maintain the virus for several days. Thus it was considered that wild-caught specimens might contain virus, and so indicate an area of virus activity.

The observations on these two species, together with a discussion of the zoogeographical affinities of Bwamba, have been published in more detail elsewhere (Haddow, 1945a).

Before passing on to the following year's work it seems worth while to sum up the main results for 1942 with reference to yellow fever epidemiology. By the end of the year it had been established that in this area at least yellow fever virus was capable of remaining active after the human population had been eliminated from the local epidemiology by a very efficient mass-vaccination programme. A. simpsoni was still capable of picking up the virus and, as monkeys appeared to be important mammalian hosts of the virus - possibly the only local hosts apart from man - this mosquito probably became infected by biting monkeys during their raids on plantations. On the other hand, while A. simpsoni might be a good vector of the infection from monkey to man, or from man to man, it could hardly be an effective vector from monkey to monkey, as it entered only the extreme edges of the forest. This was confirmed by the fact that some monkey species, known as a result of protection tests to be involved in the local epidemiology, never left the forest, and in fact hardly ever entered the areas within which A. simpsoni could be taken biting. In addition, it was found that the range of movement of most monkey species in the forest was very limited indeed. The presence of immune bodies in the sera of such monkeys indicated that almost certainly there must be a monkey-to-monkey cycle in the forest, transmitted by some arthropod other than A. simpsoni. It seemed certain that A. aegypti, the classical vector of the disease, was unlikely to play any important part in the local epidemiology of the infection in man as it was not common as a domestic mosquito, though the larvae were not uncommon in tree-holes and were also sometimes found in plant axils. This mosquito, in fact, seemed to be present in what might be described as the wild state.

Work carried out during 1943

During 1943 knowledge of the interior of the main forest was built up rapidly. Nine journeys to the Semliki River were made and, through collaboration with the Forest Department, a number of cut lines forming a survey grid were prepared. These were plotted with chain and compass, and the forest map began to take more detailed shape.

Similarly, where mass catching was concerned, work was extended far beyond the study area originally selected. Thus the previous year's monkey sample had shown that yellow fever virus was active in various forest areas which had previously been left unstudied as they were uninhabited and as ideas concerning the zones of virus activity had, till then, been based entirely on surveys of immunity in man. Catches were now, therefore, made in many localities - both inhabited and uninhabited - along the edges of the main forest and along the banks of rivers running through it. By the end of the year three large catches had been carried out as far afield as the banks of the Semliki River, each of these yielding very considerable numbers of mosquitoes. The species list for the area was considerably lengthened and during the year 31,042 mosquitoes and 288 Tabanids were sent to Entebbe for inoculation into animals. Yellow fever virus was not obtained during 1943, but two previously unknown viruses were isolated from mosquitoes.

During January an unusually dry spell of weather cut down mosquito populations severely, and the only locality in the original study area where they could be found in any abundance was a swampy belt of relict forest at Ntaya in the Manjuguja Valley. Here a short catch was made and a virus, later named Ntaya virus, was isolated from the mosquitoes taken. A note on this agent and its isolation will be found in Appendix 3.

Later in the year a most interesting virus, later known as Bunyamwera virus, was isolated from mosquitoes caught in forest on the banks of the Semliki River. A good deal of work was done on the distribution of this agent in Bwamba. A summary of this, together with notes on Bunyamwera virus and its isolation, is given in Appendix 4.

Where mammal work was concerned less time was devoted to general collection during 1943, only specimens of special interest - 34 in all - being sent to Entebbe. On the other hand, the collection and observation of monkeys was greatly increased, and during the year nine were sent to Entebbe alive while 95 were shot for the collection of serum and other study. Tests were carried out successfully on 84 of these monkeys. Thus, since the beginning of 1942 a total of one infant monkey (see below) and 97 others had been tested, and among these were 68 immune to yellow fever. This was a most significant observation as the immunity rate, 68/97 (or 70 per cent) was extremely high - much higher than in any human population tested in Africa up to that time. Though small collections had been made before by Findlay, Stefanopoulo, Davey and Mahaffy (1936), this was the first large systematic collection made from a single area anywhere in Africa, and it came as a surprise that the incidence of immunity among these monkeys should be so much higher than that in the local human population. These results led finally to an altered concept of the epidemiology of yellow fever in Africa, as follows:

It had long been considered that yellow fever was originally an

African disease. Thus in African monkeys only a mild illness - often inapparent clinically - follows infection (Bauer and Mahaffy, 1930; Smithburn and Haddow, 1949a), whereas numerous workers had shown that in South American and Asiatic species a fatal result is the rule. There may be a somewhat similar effect in man as, while deaths may occur in a high percentage of African cases during an epidemic with rapid man-to-man spread, the African at other times seems to undergo a rather mild infection (Beeuwkes, 1936; Kerr, in Strode, 1951). This again may indicate a selective process working over a very extended period. Similarly the classical vector, A. aegypti, is almost certainly an African mosquito (though it is now distributed widely in the tropics and subtropics) as only in Africa can it be found in a "wild" state, breeding in tree-holes and plant axils, sometimes in the complete absence of man. Elsewhere, notably in South America, it is rigidly domestic and seems to be unable to support itself in forest, even when artificially introduced to that environment in large numbers (Shannon, 1931). It has been suggested, as mentioned above, that yellow fever virus invaded the Americas in the slave ships, which also introduced the mosquito vector. This view was supported by the monkey sample now under discussion. Thus in South America epizootics sweep through the monkey population, killing large numbers and immunising a very high percentage of the relatively few survivors. After such an episode the disease moves on through the forest and does not reappear in the same area for a long time, as it takes years to build up from the few survivors a large enough susceptible population to support another epizootic wave (Bugher, Boshell-Manrique, Roca-Garcia and Osorno-Mesa, 1944). The Bwamba sample showed an entirely different process to be in action. Though the actual ages of the specimens were not known, study of weights, measurements, pelage, genitalia, and particularly of the order of eruption and replacement of the deciduous teeth and of wear in the permanent teeth has allowed subdivision of the sample into age-grades. The recognition of these grades has been discussed in greater detail elsewhere (Haddow, 1952a) and reference should be made to this paper for detail. They agree broadly with those given by Gilmore (1943) for a South American species. The grades used at the Virus Research Institute are:

1. Infant. Birth to about six months. This grade was not at first recognised as separate from the juvenile grade, and in the first paper on the subject (Haddow, Smithburn, Mahaffy and Bugher, 1947) the two grades were combined.
2. Juvenile Six months to about one and a half years.
3. Subadult One and a half to about three years.
4. Adult Three to about ten years.
5. Old Over ten years. The maximum in nature is probably about twenty years.

When the sample was subdivided into these grades the results were found to be as follows:

Infant	1/1**	-
Juvenile	0/15	(0%)
Subadult	5/12	(42%)
Adult	52/60	(87%)
Old	10/10	(100%)

The numerator shows the number immune, the denominator the number tested.

** This monkey was listed as a juvenile in the first paper referred to above.

At this point it may be noted that infant monkeys are usually excluded from considerations of the incidence of immunity. This is because the offspring of immune mothers - both monkey and human - are born with a transitory passive immunity which lasts for at least six months (Hoskins, 1934; Soper, Beeuwkes, Davis and Kerr, 1938). Thus the immune infant noted above is considered as more likely to have derived its immunity from the mother than from an attack of the disease. The rest of the sample under discussion shows that the incidence of immunity increases with increasing age. In South America, where a typical epizootic situation exists, one would expect that after an outbreak a very high percentage of the survivors would be found to be immune and that the incidence of immunity would be almost unrelated to age. On the other hand, in the pre-epizootic phase, one would find immunity only among the oldest monkeys, survivors of the last epizootic wave. The above figures, however, present a clear and typical picture of enzootic disease, where all age-grades are equally exposed throughout life to an infection either constantly present or else occurring in small often-repeated waves without a major outbreak. Additional facts helping in the appraisal of the situation were that (a) immune monkeys were to be found throughout the lowland forest areas (as had been shown by tests on specimens collected along the edges of the Semliki Forest, on others collected in its gallery extensions and outliers, and on yet others collected in the forest interior during journeys to the Semliki River), and that (b) all the lowland monkeys seemed to be involved in the epidemiology, as immune specimens had been found in all six lowland species tested up till that time.

The facts that in this first large sample of African monkeys the incidence of immunity was much higher than in the human populations, that all the lowland species which had been sampled were involved, that the disease is rarely fatal and seldom severe in African forest species and that the infection was clearly enzootic, led to the altered concept mentioned above. Thinking had been coloured by the serious nature of the infection in man in instances where epidemic man-to-man spread of the "yellow jack" type occurs. Now, however, it seemed clearly indicated that not only were African monkeys important mammalian hosts of the virus but that they should almost certainly be regarded as the natural hosts (Haddow, Gillett and Highton, 1947). Yellow fever, therefore, in what is probably to be considered its real home, the African rain-forests, should really be considered as a mild enzootic infection of monkeys which from time to time "escapes", as it were, into the human population, more or less by chance. Around this view much of the subsequent work in Bwamba and elsewhere has been built up.

The observation of monkey bands was now obviously important and an increasing amount of time was spent on this study. One of the main findings was that the common species had regular sleeping-trees in which they assembled shortly before sunset (Haddow, Smithburn, Mahaffy and Bugher, 1947; Haddow, Gillett and Highton, 1947). This subject was later studied in more detail by Lumsden (1951b) and by Buxton (1951).

It is of interest to note that some of the sleeping-trees noted by the writer in the year following that at present under discussion (i.e., in 1944) were still in use when Buxton made his observations in June, 1950. The importance of the sleeping-trees in relation to yellow fever transmission among monkeys will appear below.

Though it was now considered as established that monkeys were the natural mammalian hosts of yellow fever, and though the study and collection of other mammals had been cut to a minimum, it was felt that some other vertebrate host might exist. Thus when a monkey experiences an infection it circulates virus for a few days only. Shortly afterward, it has become solidly immune, and the immunity persists for life. It is, therefore, unable to play any further part in maintaining the virus. As even in the places where they are most numerous the numbers of monkeys are never of the order shown in populations of some of the smaller vertebrates, the question arose repeatedly as to whether some of these might be involved in the picture, acting as a reservoir of infection. In this connection the work of Sawyer and Frobisher (1932) on the American bull-frog, Rana catesbiana, seemed of interest. This frog is useful in the laboratory as, when placed in a refrigerator, it enters hibernation and requires little further attention. Sawyer and Frobisher showed that, though virus could be recovered from bull-frogs up to 4 days after inoculation, antibodies were not developed, and that if a second inoculation were given later, there was once again some persistence of virus. Stefanopoulo and Nagano (1937) were unable to repeat this observation with Rana temporaria kept at room temperature, but it was felt that further studies were indicated, as it was considered that cold-blooded animals, which exist in enormous numbers in tropical rain-forest, might possibly act as purely passive reservoirs.

Considerable numbers of frogs (particularly tree-frogs and axil-dwelling species), toads, geckos, tortoises and snakes were collected both in Bwamba and at Entebbe. Identification was very difficult, as the literature was out of date, and as various species not recorded from East Africa were obviously present, in addition to others thought to be undescribed. Techniques were worked out for bleeding these animals and for feeding mosquitoes on them. It was found that some mosquitoes would feed readily on frogs and geckos (and vice versa). Many of these cold-blooded animals were inoculated directly with the virus, and others were bitten by artificially infected A. aegypti. Large numbers were bled for protection test. No immune specimens were found and none of the artificially infected specimens either circulated virus or showed immunity subsequently. These animals, in short, appeared to be completely resistant to infection with yellow fever virus, and the result obtained by Sawyer and Frobisher was concluded to be due to the fact that the frogs studied had been kept in a refrigerator in a hibernating state. In the following year Laemmert (1944) who, unknown to the Entebbe unit, had been carrying out very similar work, published results indicating that South American poikilothermic animals were completely resistant to infection. The work at Entebbe, which had been carried out by Smithburn, Mahaffy and the writer in equal collaboration, was then discontinued and was not published in detail, though it is briefly referred to by Bugher (in Strode, 1951).

Investigative work on mosquitoes in 1943 was almost completely concerned with 24-hour catches and with work on the microclimate of mosquito environments. The latter was hampered by the fact that only two sets of recording instruments (thermographs and hygrographs) were available and, for the duration of the war, it was not possible to obtain others. Thus instead of making a simultaneous comparison between the environments which it was desired to study - the open air, the plantations and the forest - it was necessary to compare open air (the control) with plantations, then with forest, and finally plantations with forest.

The instruments were exposed in identical Stevenson screens, at a height of three feet above ground-level and the screens were never more than 300 yards apart, as climatic incidents such as gusts of wind and small rain showers are often exceedingly localised in equatorial countries. Light observations were made with the only photometer available, a small photographic one, calibrated in arbitrary units. The method here was to place an inverted bowl on a stake 3 feet high and to cover it with a white cloth to give a dazzle-free surface. The reading was taken 6 inches from the most brilliantly illuminated side, the axis of the photometer being perpendicular to the surface of the bowl, and therefore always pointing slightly downward. Thus direct sky light could not enter to affect the reading. The runs for the thermographs and hygrographs lasted for a week each. In the case of the light readings, these were taken hourly at each of the pair of stations concerned, the run lasting for 96 hours in two cases and 48 hours in the third (the plantation-forest comparison). As the results have been discussed fully elsewhere (Haddow, 1945b) only the main points need be covered here.

By day the climate in the open showed rather low relative humidity (hereafter R.H.), fairly high temperature and saturation deficiency (hereafter S.D.) and intense light. In banana plantations the R.H. was distinctly higher, temperature and S.D. lower, and light less intense. In forest R.H. was very high, temperature still lower, S.D. very low indeed and light of very low intensity. Small short-duration fluctuations in the traces - very apparent in the open-air screen recordings - were very much reduced in the plantation traces and hardly apparent in those taken in forest. These small fluctuations are caused by such minor incidents as puffs of wind, passing clouds, etc. Generally speaking, the range of the various readings was great in the open air, small in banana plantations, and very small in forest.

An important point concerned the duration of the more rigorous conditions of the daytime in the various environments. Thus where 25°C. was taken as an arbitrary level, it was found that in the open this temperature was exceeded for about 8 hours in one series and 9 hours in the other. In plantations the corresponding figure was just under 6 hours in one series and just over it in the other. In forest the figures were only 2 hours in one series and 3 in the other. In the case of relative humidity the results were even more striking. Here 70 per cent R.H. was taken as the arbitrary level. In the open air stations it was found that in one series R.H. fell below this level for about 6 hours daily and in the other for about 7 hours daily. The corresponding plantation figures were 2 and 3 hours. In the forest the mean R.H. did not fall as low as 70 per cent at any time. In the case of S.D., which gives an index of the drying power of the air, 7.0 mm. was selected as the critical level. This was exceeded in the open air for 7 hours in one series and 8 in the other. In plantations the corresponding figures were 3 and 4 hours. In one series the forest reached 7.0 mm. for a mean of one hour daily. In the other a mean of 7.0 mm. was not reached in this environment. Where light was concerned the following points are of interest: In the comparison between open and plantation, light in the open had reached by 07 hours a level as high as the whole day's maximum for the plantation and, in the comparison between open and forest, light in the open had reached by 06 hours a level twice as high as the forest day maximum. Finally in the comparison between plantation and forest, light in the plantation had reached by 06 hours a level higher than the forest day maximum.

The picture which thus emerged was not unexpected, though in some cases the differences between the environments were surprisingly large. This is not shown so well by mean figures as by the absolute maxima, minima and ranges, for which reference should be made to the paper quoted above. Broadly speaking, the open air showed a good deal of diurnal fluctuation in all factors here discussed. The plantations were cooler, moister and more equable. The air above the forest floor was still cooler and moister and here the equability was of a very striking degree indeed, as is shown by the following figures: In the two weeks' readings the mean temperature range 3 feet above the forest floor was only 7°C. (18.5 - 25.5°C.), the mean range of relative humidity was only 20 per cent (71 - 91 per cent) and the mean range of saturation deficiency was only 5.5 mm. (1.5 - 7.0 mm.). Thus this environment was never cold and the air never had much drying power. These readings were taken during what was, for Bwamba, relatively fair weather. Other series taken subsequently, during the rains, showed even smaller ranges for all factors, relative humidity in particular being near saturation at all hours in the forest. In the rains, in fact, the space above the forest floor formed a sort of natural "controlled conditions room", except, of course, where light was concerned.

There were one or two unexpected results as follows: Where temperature and atmospheric humidity (and to a smaller extent, light) were concerned, it was found that in plantations the conditions of the later night hours persisted for some time after sunrise, and this was even more marked in forest. Thus it was not surprising to find that certain nocturnal mosquitoes, whose activity ends at sunrise in the open, may continue to bite for some time thereafter in plantations, and even longer in forest. Thus a non-domestic species, which in the open bites by night only, may never make contact with man in that environment. In plantations and forest, however, it may bite man just after sunrise, a period of marked activity both in the case of the peasant farmer and of the hunter.

The other unexpected finding concerned the night hours. Most of the discussion above concerns the wide differences found between the various environments by day. By night, however, these were very greatly reduced and in consequence it might be said that at this time the micro-climatic barriers between these environments broke down. Infiltration of banana plantations by forest mosquitoes had been noted, and it now seemed likely that this took place at night and further, that both forest and plantation species might at that time invade open ground and areas around huts. It will be remembered that work at Kisumu (Haddow, 1942a) had already shown that "wild" species such as T. africanus may enter huts by night in considerable numbers, bite, and leave before sunrise, their true prevalence in this environment remaining unsuspected unless night catches are made.

The first series of 24-hour catches consisted of 15 made in forest, and 15 in banana plantations. Altogether 36 species and groups of mosquitoes were taken. The details of the methods and the results have been discussed fully elsewhere (Haddow, 1945b) and in the present communication only a few of the main points can be picked out for discussion. All these early catches were made with a group of unskilled people acting as baits, while the actual collection of mosquitoes coming to bite was done by experienced catchers.

Generally speaking the patterns of biting-behaviour - which were now termed the biting cycle - were repetitive from day to day, though in a few cases, such as Aëdes circumluteolus, a clearly defined cycle was not shown. Two main types of activity occurred. In the first there was one main period of activity in the 24 hours, this occurring by day in some species and by night in others. In the second type of cycle there were two main periods of activity, and these usually occurred around the times of sunset and sunrise. The term "eo-crepuscular" was introduced to describe this type of activity. Another type of biphasic activity later observed in many day-biting species shows a peak of activity during the morning, a lull around noon, and a second peak of activity in the afternoon. It was obvious, from an early stage, that the 24-hour catch was an effective method of studying biting activity, and now for the first time it became possible not only to search for a given mosquito in the most favourable environment, but also to concentrate on the period when the largest numbers would be taken biting.

In working out the results of the climate observations mentioned above, it had been noted that the 24 hours fell naturally into six 4-hour periods, each with microclimatic characteristics differing from those of all the others, as follows:

Period 1	06-10 hours. Sunrise and morning. Very high R.H., low temperature and S.D. Light intensity low at first.
Period 2	10-14 hours. Middle day. Minimum for R.H., maximum for temperature, S.D. and light.
Period 3	14-18 hours. Afternoon and evening. Low R.H., fairly high temperature and S.D., light decreasing.
Period 4	18-22 hours. Sunset and early night. Very high R.H., temperature rather lower, and S.D. very low. Light readable only for a short period after sunset.
Period 5	22-02 hours. Middle night. Very high R.H., temperature falling, S.D. very low.
Period 6	02-06 hours. Late night and dawn. Very high R.H., temperature at a minimum and S.D. very low. Readable light appears a little before sunrise.

In East Africa a type of "summer time", known as East African Standard Time, is used for everyday purposes. In these catches, however, it was necessary to use Local Mean Time (i.e., Greenwich Mean Time with a correction for longitude). Using this measure, sunset at the equator (as in Bwamba) occurs at 18 hours ± about 17 minutes. Sunrise occurs at approximately 06 hours, once again with a small range of variation during the course of the year. Local Mean Time was used throughout the writer's earlier work on biting cycles, but at a later date Lumsden (1952) pointed out that for detailed work it was preferable to relate the catch time to the actual time of sunset, using a "Catch Time" in which the clock is adjusted in each catch so that sunset will occur exactly at 18 hours.

In these first catches, though the mosquitoes taken were grouped by single hours, a broader grouping was used in the interpretation of the results, and it was found convenient to use the same 4-hour grouping as has been described above for microclimate. Thus if a species showed a peak of biting activity of short duration after sunset with only small numbers thereafter till a similar but smaller peak occurred before sunrise, it could be argued that the mosquito concerned bit at times of low (crepuscular) light intensity and high humidity but that, while sunset was a period warm enough to permit free biting activity, dawn was too cold. Such a basis for argument has remained useful up till the present, and the suggestion that biting cycles might be controlled by microclimate was subsequently accepted both by De Meillon (1947) and Bates (1949). In the case of A. gambiae, for example, the biting-cycle as shown in forest in Bwamba was closely comparable to the entry cycle found in huts at Kisumu, with a low level of activity during the day, an abrupt onset of biting after sunset and a gradual increase throughout the night to a well-marked maximum shortly before sunrise. During the night, the period of main activity, humidity remains very high, and the drying power of the air is very low throughout. On the other hand, temperature falls steadily from sunset till sunrise, and it was felt that in A. gambiae biting activity and temperature might be inversely related. Muirhead Thomson (1951) has, however, pointed out that the range of temperature involved was very small and that this might be a case of "correlation without causation". Looking back on these and subsequent results the writer feels that Muirhead Thomson's comment may well be correct, but not necessarily so. It is interesting to recall that the same worker found in experiments with Culex fatigans that this species was exceedingly sensitive to temperature in preference chamber experiments, and it seems appropriate to quote part of his summary of this work:

"The most striking feature of behaviour at all stages was the strong avoidance of high temperatures. This was strongest in the hungry females, less strong in the blood-fed females and those with mature ovaries, and least strong in the newly emerged females.

"Newly emerged females showed avoidance of high temperature below 30°C. but not below 25°C. They also showed a weak avoidance of low temperature. At 29°C. they were sensitive to a difference of 1°C or a gradient of 0.05°C. per cm." (All underlining in this quotation by A. J. H.).

"Hungry females showed a strong avoidance of high temperature below 25°C., the reaction still taking place below 15°C. There was no avoidance of low temperatures.

"Blood-fed females and those with mature ovaries showed a strong avoidance of high temperatures below 25°C., but below 20°C. they were unaffected by temperature differences. Blood-fed females were sensitive to a difference of 1°C. or a gradient of 0.05°C. per cm. at 23°C.

"Except in the case of newly emerged females there was quite a close relation between the reactions to temperature and the effects of temperature.

"Reasons are given for regarding the temperature reactions of Culex as of first importance in determining the behaviour of the mosquitoes when seeking a resting place". (Muirhead Thomson, 1938).

Doubtless each mosquito differs in its preferences, but the above results show that the behaviour of one species may be influenced by a difference of as little as 1°C. (in the Bwamba catches the mean nocturnal range in forest was about 4°C. and in plantations about 5°C.). Further, if temperature may have a profound influence on the selection of a resting place it may have an equally profound influence on other activities, such as biting.

An aspect of the possible temperature responses of the mosquito which, it has recently seemed to the writer, has not received adequate attention, concerns the temperature of the host. That, within a certain temperature range, warmth does attract mosquitoes, has been shown in the laboratory by Peterson and Brown (1951) and the field by Brown (1951). Brown's field experiments, with human-type robots are particularly important as the warm and cool robots were exposed simultaneously and within a few feet of one another. Now while the normal daily range of human temperature varies only slightly (roughly ± 1°F.), it does in fact reach its lowest level at about 04 hours and is low at sunrise (Lovatt Evans and Hartridge, 1945). At sunset the human baits are, on a fair evening, warm, perspiring slightly and comfortable. At sunrise they are (subjectively) cold and miserable with chattering teeth and with skins (objectively) cold to touch. In the case of monkeys this state of affairs is even more pronounced. At night monkeys huddle together in their sleeping trees for warmth but single monkeys lose heat rapidly, even indoors. Temperatures taken recently by the writer and Dr. M. P. Weinbren in captive monkeys confined singly in cages at Entebbe have shown that by night the temperature may fall as much as 5-6°F. (say 3°C.) and a rapid fall also occurs in anaesthetised monkeys (Haddow and Dick, 1948). Monkeys do not become active until the sun is well up (Haddow, 1952a) and around the sunrise period they are miserable and sluggish with the fur fluffed out to the maximum. In catches with human and animal bait these facts may well play a part in determining the attractiveness of the host.

Though the writer prefers to keep an open mind on the subject just discussed, he now feels that the treatment of the climate and mosquito figures by 4-hour groups oversimplified what has since proved to be an exceedingly complicated matter. Though this broad grouping was useful in indicating general trends of behaviour, particularly where the samples were small, it naturally obliterated small peaks - some of them very constant - which have since been the subject of much study and discussion (Lumsden, 1952; Haddow, 1954).

Among the other results of the 24-hour catches a point of interest concerns the relation between the mosquito fauna of forest and that of plantations. It was known, firstly, as a result of routine catches and, secondly, as a result of the 24-hour catches that while most species can be taken throughout the 24 hours almost all are either preferentially nocturnal or diurnal as the case may be, very few species being equally prevalent by day and by night. Similarly while many were to be found both in plantations and forest, almost all showed a distinct preference for one environment or the other. In the thirty catches under discussion the number of species taken in forest by day (28) was about the same as that taken by night (29). In plantations, however, while only 14 species were taken by day, 22 were taken by night. As mentioned above, it had been concluded that by day forest mosquitoes are confined to that environment by a microclimatic barrier, but that with the breakdown of the barrier by night they may diffuse into plantations. It was considered that the above facts supported this view, more particularly when the following details are taken into account: In the thirty catches 22 species were common to both environments. When, however, the results were subdivided into day and night periods, it was found that by night 20 species were common to both, while by day only 12 were common to both. This does not, of course, imply that forest mosquitoes are not present in plantations by day, but merely that they are not biting actively in this environment at that time. Thus it is suggested that they diffuse into plantations by night when conditions are favourable, but by day are at least partly inhibited from biting, possibly by adverse microclimatic conditions.

At the time it was felt that this nocturnal invasion of plantations by forest mosquitoes might well be the link between the forest and human cycles of yellow fever. Work in subsequent years, however, showed that it is probably of minor importance in this connection in Bwamba, though it may be important in the case of other localities (Gillett, 1951a).

One particularly interesting 24-hour catch was made where the main road at Kanyamwerima is bordered by banana plantations on the one side and by dense forest on the other. The catch was made simultaneously in both environments, one bait group being within the edge of the plantations and the other - comparable in number - within the edge of the forest. The two units were only 40 yards apart. The results, however, showed wide divergence between the two environments. The forest unit took 106 mosquitoes, belonging to 16 species, while the plantation unit took only 74 belonging to 10 species, in spite of the fact that the plantation was very jungly and overgrown. In the plantation catch A. simpsoni made up 23 per cent of the total. In the forest only one specimen was taken, less than 1 per cent of the total yield. This emphasizes how scarce A. simpsoni is even within the extreme edge of the forest, and even when a large plantation is contiguous. In this double catch 10 species were common to both environments, and the remaining 6 occurred in the forest only. Thus none were peculiar to the plantation. This method of simultaneous 24-hour catching in different environments for the sake of comparison was later developed by Lumsden (1951a), whose interesting results, obtained in Bwamba, confirmed the very sharp differences between the mosquito populations of different environments, even when the catching units are only a few yards apart.

Since these first series were made, the 24-hour catch has remained the writer's main line of approach to the question of mosquito biting-behaviour. The work is of a laborious and tedious nature, as adequate personal supervision is of the utmost importance. The method was slow to find acceptance, probably on account of the necessity for being up and out-of-doors at all times of night, but it has gradually been taken up by other workers at Entebbe (Lumsden, 1951a, 1955 a and b; Lumsden and Buxton, 1951; Gillett, 1951a) and elsewhere (Mattingly, 1949 a and b; Deane, Causey and Deane, 1948). Mattingly's results are of special interest as they show, from catches made in Nigeria, that the biting cycles of various common species are closely comparable to those of the same mosquitoes in Bwamba, approximately 2,000 miles away.

Towards the end of 1943, just before these series had been completed, a progress note was received from the Rockefeller unit in Colombia which completely changed the outlook on yellow fever epidemiology in the forests. Actually this note reported work carried out almost two years before. This was, however, conducted in remote country and confirmatory studies were carried out before the note was prepared and circulated. Yellow fever virus had several times been isolated in South America from mosquitoes of the genus Haemagogus, but these mosquitoes appeared most irregularly in the catches, and could not be found at all in dry weather.

It was thus considered that "carry over" of virus from one season to the next was not likely to be effectively carried out by Haemagogus spp. The explanation came when a party led by Dr. Jorge Boshell-Manrique, a most gifted and enthusiastic field naturalist, stopped to watch some woodcutters felling a tree during the dry season (in South America "jungle yellow fever" usually appears first among woodcutters working in the main forests). After the fall of the tree Haemagogus mosquitoes at once appeared in numbers, biting actively, and it was realised that they had been present in the crown of the tree. Dr. Boshell has told the writer that he had suspected some such state of affairs before this incident, but had not made any investigations. When he noticed the tree being felled he saw an opportunity to put the matter to the test. Work followed rapidly to show that Haemagogus spp. and other mosquitoes are prevalent in the canopy and can be taken there at all seasons (Bugher, Boshell-Manrique, Roca-Garcia and Osorno-Mesa, 1944; Bates, 1944). It is extraordinary that both in South America and in Africa this simple explanation to the problem was missed for so long. Thus in both areas it was considered that monkeys were the principal mammalian host, and it was known that in both areas a high incidence of immunity might occur in species which seldom if ever descend to ground level. No one, however, made the simple deduction that obviously the vector mosquitoes of the animal-to-animal cycle must also, therefore, be arboreal till Boshell's fortunate observation gave the clue.

To sum up the main results of work in Bwamba in 1943 where yellow fever is concerned: It was considered that the relationship of the monkey population to the infection had been established and that they were almost certainly the main mammalian hosts of the virus and quite probably the natural hosts. On the other hand, the reptiles and amphibia were regarded as eliminated from further consideration. A good deal of study had been carried out on the mosquitoes of plantation and forest and the 24-hour biting cycle of some of the commoner species had been worked out, the biting habits being studied particularly in relation to microclimate. Finally, the study of sera collected from monkeys had showed that the infection was prevalent throughout the lowland forest, even in areas 6-7 miles from the nearest human habitations.

Work carried out during 1944

When the progress note from South America was received it was not feasible to change the programme immediately and, though the matter obviously called for investigation at the earliest possible date, nothing could be done till 1944. A serious difficulty was, however, that the Baamba (though a forest tribe) are poor climbers, and the staff were not at all willing to begin work in the trees. Two short trial catches of about an hour each were, however, made by day. One of these yielded no mosquitoes. During the other, 2 Aëdes apicoargenteus were taken biting at a height of over 20 feet, and there thus seemed a clear indication that at least some activity was going on well above ground level. It was felt that the best approach would be to begin work on very low platforms, to accustom the catchers to the idea, and it so happened that this fitted in well with another section of the work, as follows:

When monkeys raid a plantation, they usually race in through the undergrowth, and at such a moment are not likely to be bitten by A. simpsoni. They then climb the largest plants, where the fruit is almost ripe, and sit there eating the bananas. From a distance one can tell which plants are being attacked by the swaying of the leaves (it seems worth noting that shaking the vegetation is a well-known device of mosquito catchers to bring mosquitoes out to bite). The question was whether A. simpsoni was present and active well above ground level among the leaves of the larger plants, and it was decided that this could best be investigated by means of low platforms.

Three platforms were accordingly built in a large banana plantation, at heights of 6, 12 and 18 feet above ground level (all in one structure). The highest was above even the largest plants, the next among the leaves of the full-grown bananas, and the lowest among the leaves of the young bananas, but still well above the undergrowth. The ground level control station was at the foot of the platforms, in the undergrowth of colocasias and grass.

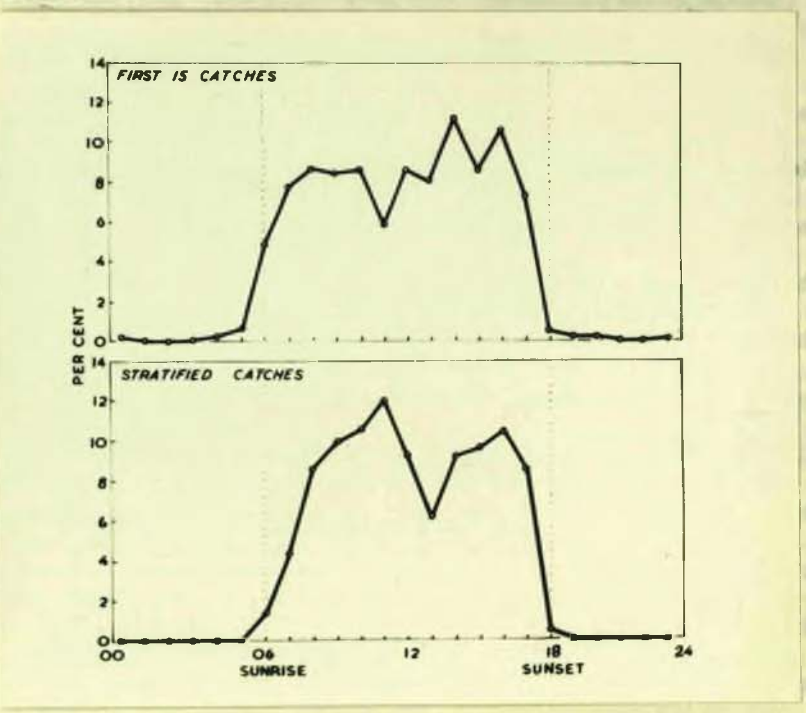


Fig. 2

The biting cycle of Aëdes simpsoni. Top figure - the original 15 catches in banana plantations. Bottom figure - the 10 stratified catches, all levels collectively. The figures are geometric means, shown on a percentage basis to facilitate comparison.

A series of ten 24-hour catches was made early in 1944, catching being carried out simultaneously on all three platforms and at ground-level. Altogether 16 species and groups of mosquitoes were taken, totalling 687 specimens, of which 596 (87 per cent) were A. simpsoni. The main results may be summarised as follows:

Level	No. of mosquitoes	No. of A. simpsoni	No. of species
18 feet	128	117	6
12 feet	167	141	7
6 feet	182	159	9
Ground level	210	179	11

Thus the total yield, the yield of A. simpsoni and the number of species taken all decreased, though not spectacularly, with increasing height. A. simpsoni was obviously quite common at all levels in the plantation and also in the open, above the largest plants. Thus raiding monkeys attacking bananas are liable to be bitten by this species even in the crowns of the full-grown plants.

During this series it was found that in bright, hot weather (usually cold in the morning) the numbers taken biting were much smaller than on days when a warm, humid morning was followed by an overcast day - warm, but without bright sunshine. This was in agreement with previous observations by Gibbins (1942) and the writer (1945b). The general form of the biting-cycle was similar in both cases, but in hot bright weather when the morning was cold, biting activity began later.

It is convenient to discuss at this stage the biting activity of A. simpsoni, as the work done on this species in later years consisted mainly of surveys, etc., and little deliberate study was carried out on it after 1944, with the exception of the plant axil work discussed above.

In the first series of 24-hour catches in banana plantations, it was found that A. simpsoni is almost entirely diurnal. The main periods of activity were in the morning and afternoon, and there was a partial intermission during the hot part of the day. In the paper describing these catches (Haddow, 1945b) only the 4-hour grouping was published. The hourly results (Fig. 2) are now shown for the first time. The curve

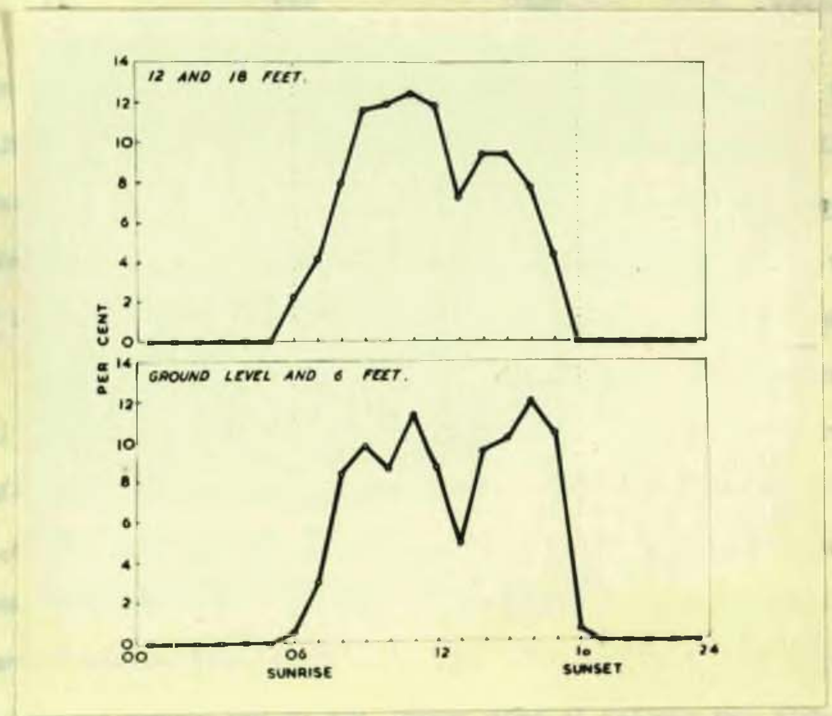


Fig. 3

The biting cycle of Aëdes simpsoni, as shown in 10 stratified catches. Top figure - the combined cycle for the 18-foot and 12-foot platforms. Bottom figure - the combined cycle for the 6-foot platform and ground level station. The figures are geometric means, shown on a percentage basis to facilitate comparison.

is of the diurnal biphasic type first described by Kumm and Novis (1938) in the case of two mosquitoes of the Amazon Valley which they studied by means of day catches. In this first series the intermission occurred in the period 11-12 hours. In the second series, the ten stratified catches discussed above, the cycle was similar, but the intermission was much later, occurring in the periods 13-14 hours. This curve has been published in the paper discussing these catches, but only on an arithmetic basis. It is shown again here, using the more reliable geometric mean, following the method of Williams (1937). It is interesting to note that a biphasic rhythm of this type may occur quite apart from biting, as Johnson and Taylor (1955) have found a very similar pattern in the flight activity of a non-bloodsucking insect, Aphis fabae. When the results for the various levels are examined separately, it is found that in each case the partial intermission occurs at the same time and that all the curves are biphasic. In the paper describing these catches (Haddow, 1945c) the results for the separate levels are shown on a 4-hour basis and in this rather coarse grouping the biphasic nature of the curves is lost. The main point to be noted is that at ground level and at 6 feet the afternoon peak was the more pronounced, as in the original series of 15 catches in plantations. At 12 and 18 feet, however, the afternoon peak was much reduced, and this was considered to be almost certainly due to the fact that in Bwamba a breeze blows from the mountains in the afternoon with great regularity. This breeze affects the upper levels of the plantation much more than the lower levels. The biting-cycles for ground level and 6 feet (combined, as they are very similar) are shown in Fig. 3, where also will be found the combined cycle for 12 and 18 feet (also very similar). In plantations, therefore, there seems to be a good deal of activity at all levels throughout the day.

Some years afterward, Lumsden (1955b), working in the Taveta forest in Kenya, found that A. simpsoni was here very prevalent in the forest itself. This was completely different from his own experience in Bwamba (1951a, 1952) and also from the writer's experience in Bwamba and elsewhere. The reason was probably that at Taveta the bananas are grown

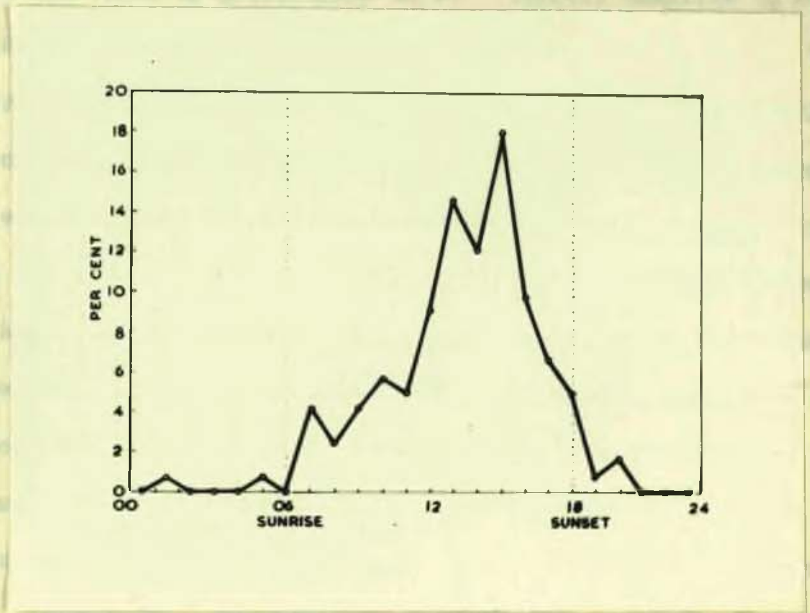


Fig. 4

The biting cycle of Aëdes simpsoni in forest. The figures are geometric means, shown on a percentage basis to facilitate comparison.

actually in the shade of the forest trees in small patches and clumps. Both in the forest and in the banana plantations the cycle was quite different from that found in the Bwamba plantations. Thus in the morning there was very little biting. There was a further drop in activity at about 11 hours, and the main wave of activity came in the afternoon.

In the first series of 24-hour catches made in forest in Bwamba, only 23 A. simpsoni were taken among a total of 2,185 mosquitoes. Most of these bit during the afternoon. Subsequently an extensive series of 24-hour catches was carried out, 240 catches (six each at 40 different stations in the Semliki Forest) being made in all. This series (the Bwamba Tree Survey) will be discussed further below. For the moment it may be noted that of a total of 40,976 mosquitoes and other biting Diptera taken, only 89 were A. simpsoni. Here again the same characteristic curve appeared, with very little activity in the morning, and a major wave in the afternoon. As the behaviour of the mosquito in the first forest series and in the Bwamba Tree Survey is obviously similar, it has seemed permissible to combine them (Fig. 4) to obtain a total sample of 112 specimens taken biting in forest. The curve now obtained is strikingly similar to that obtained by Lumsden at Taveta.

The cause for this difference in behaviour - the biphasic curve in plantations and the single major wave in forest - is not clear. It might be argued that, as the plantations are the main breeding-place, the population there might contain a higher percentage of young females and that these would form the main morning wave, the older females building up the afternoon wave. In forest further from the breeding-places, newly emerged females might be fewer, their scarcity accounting for the smallness of the morning wave. Lumsden's Taveta results, however, seem to discount such a view. As well as his catches in forest he made others in banana plantations, and here the curve was exceedingly similar to that obtained in forest. The forest area at Taveta is exceedingly small, being supported mainly by springs and seepages in an otherwise rather hot and arid area. Bananas can thrive only within this small forest, which is full of tiny scattered plantations, hut clearings, etc. One is, in fact, always within a few yards of a cultivated plot, and there are also many abandoned, semi-wild banana patches. Thus breeding-places abound throughout the area, and yet both environments show the type of curve with only one major activity wave. Thus this type of curve must be taken, as Lumsden (1955b) suggests, as characteristic of a particular environment, forest. It must be added that it also appears to be characteristic of banana plantations where these are surrounded by, and dominated by, the forest.

While the stratified catches in plantations were in progress, routine catches in the main forest were also going on, but on a new system. As the previous year's sample of monkeys had shown that virus was active throughout the lowland forest, it was concluded that the most logical method was to concentrate on a single area where (a) the mosquito population was a large and varied one; (b) the monkey population was also large and varied and (c) there was a high incidence of immunity to yellow fever among the monkeys. During 1944 little work was done in the forest interior, beyond some mapping, as it was beginning to be apparent that the largest concentrations of mosquitoes and monkeys, and also the most varied populations, were to be found in the fringe habitats of the forest edge, or in places where a stream carried forest-edge conditions into the interior. The area chosen, Mongiro, was such a forest-edge locality, and was accessible by road. It consists of a block of dense swamp-forest, connected by a narrower strip to the main forest. Oil palm (<u>Elaeis guineensis</u>) and wild date (<u>Phoenix reclinata</u>) predominate, together with the large-leaved swamp-loving tree <u>Mitragyna stipulosa</u>. The area has, in fact, reached the edaphic climax. The forest is very jungly and 'tropical' in type, with a wealth of creepers, rattans and lianas. Mosquitoes are very abundant, and the species list is a long one. Monkeys are exceptionally numerous. In the square mile of forest at Mongiro it was estimated that there were at that time about 400 monkeys, and it was known that nine species were represented in this total. Of the 17 monkeys which had been collected there, 13 (76 per cent) had been found immune to yellow fever.

This area - very important in subsequent work - has been described in more detail elsewhere (Haddow, Gillett and Highton, 1947, where some photographs of the area are included, and Lumsden, 1951b, where there is a detailed map).

Though Mongiro is on the forest edge, the surrounding area was not inhabited at that time, as the locality was feared by the Baamba. Thus there was a former site of human sacrifice (used within living memory) at the Mbuga hot spring on one side of the area, while on the other was the deserted clearing of Bamaga, where an entire village had been wiped out by an epidemic. The intensive catching at ground-level which began here in 1944 led, during the first half of the year, to an isolation of yellow fever virus and to six isolations of Rift Valley fever virus. The latter are discussed in Appendix 5. The circumstances of the yellow fever isolation were as follows:

The year was a heavy one where mosquito collection for virus isolations was concerned, 54,125 mosquitoes being taken for this purpose, of which 35,529 were sent to Entebbe. In the fifth catch of the year, which covered the period 18-23 April (while the stratified catches in banana plantations were still in progress), 4,310 mosquitoes were caught in a dense part of the Mongiro block, and of these 3,860 were sent to Entebbe. The inoculation groups were Anopheles, Taeniorhynchus, Eretmapodites, Culex, A. tarsalis group, A. circumluteolus and "Other Aëdes". The "Other Aëdes" lot consisted of 80 insects, belonging to 12 species. They had been pooled because none were present in large enough numbers to warrant the allocation of a separate monkey and mouse-groups for inoculation. The lot was made up as follows:

A. apicoargenteus	3
A. dendrophilus***	12
A. africanus	3
A. haworthi	1
A. argenteopunctatus	4
A. mutilus	1
A. sp. aff. abnormalis	9
A. lamborni	3
A. cumminsi	13
A. natronius	11
A. palpalis	11
A. taeniarostris	9

*** Owing to an error in Hopkins (1936) this species was at that time listed as A. deboeri ssp. de-meilloni. The error, it should be noted, was not Hopkins', but occurred in a quotation from another source.

From this lot yellow fever virus was isolated. Thus at last there was conclusive evidence that yellow fever virus occurs in African forest mosquitoes in primary rain-forest uninhabited by man. The circumstances of the isolation have been described in more detail elsewhere (Smithburn and Haddow, 1946).

Now that the presence of a forest vector had been established, it was felt that certain theoretical requirements for such a vector could be listed to aid selection, from the above list, of the most likely suspects. The criteria were as follows:

(1) The mosquito must be able to transmit the virus by bite.

(2) It must have a wide distribution in tropical Africa.

(3) It must be relatively common.

(4) As strictly arboreal monkeys may show a high incidence of immunity, it must be a species which bites freely in the forest canopy.

(5) By this time it was known that in Bwamba some monkey species are strictly arboreal, others partly arboreal and others mainly terrestrial; all, however, sleep in the canopy by night. The incidence of immunity was closely similar in all three groups. This could be explained in two ways. If the vector were a mosquito biting mainly in the canopy, then it must do so at a time when all monkeys are in this environment, namely, at night. On the other hand, the vector could be a diurnal mosquito if it bit with equal freedom at all levels, namely, in the canopy, the understorey and at ground-level (it may be mentioned here that no mosquito with such a behaviour pattern has yet been found).

The basic assumption was of course that there was one forest vector over a wide area of the African rain-forest.

When these standards were applied to the mosquitoes listed above, it was at once apparent that most of them could be dismissed from further consideration. Thus on the basis of scarcity, or of restricted distribution, or of both, A. dendrophilus, A. haworthi, A. mutilus, the species near A. abnormalis, A. lamborni, A. natronius, A. palpalis and A. taeniarostris could be eliminated. Of the four remaining species one, A. apicoargenteus could also be eliminated as Bauer (1928) had shown that it cannot transmit the virus by bite. It may be mentioned that this result was confirmed by laboratory experiments at Entebbe, carried out by Dr. K. C. Smithburn, Dr. T. P. Hughes and the writer. These experiments were never published in detail, but were considered to be conclusive. Nothing was known about the capacity of A. cumminsi or A. argenteopunctatus to act as vectors. Bauer, however, had shown (loc.cit.) that A. simulans can transmit the virus under laboratory conditions. This mosquito belongs to the same subgenus (Aëdimorphus) as A. cumminsi and A. argenteopunctatus and is quite closely allied to the latter. They thus remained on the suspect list. The remaining species, A. africanus, was however considered by far the most likely suspect. Thus it had a distribution extending from West Africa to Kenya, and it is a common forest mosquito throughout this vast area. It had already been shown by Philip (1929) that it is an efficient vector under laboratory conditions. In addition, it is a member of the same subgenus as the two African vectors then known (A. aegypti and A. simpsoni). Finally, it was known, as a result of the 24-hour catches discussed above, that it has a very well-marked peak of biting-activity in the hour after sunset, by which time monkeys are asleep in their sleeping-trees. Nothing, of course, was known as yet of the vertical distribution of this species, or of the other two suspects.

It was decided that an intensive study of the forest canopy must be begun at once, as it was felt that if any of the three suspect mosquitoes showed marked arboreal tendencies, there would be good reason to suspect that it was the vector. Thus, even if virus was not obtained again at that time, future work could be planned so as to investigate and collect selectively the most probable vector.

Fortunately Dr. J. C. Bugher, who had led the yellow fever team in Colombia, happened to be visiting Entebbe, and was able to give much useful advice on the actual construction of platforms. As the work now planned was obviously going to be very heavy, and as speed was considered essential if there was to be any hope of a further virus isolation, the Medical Departments of Kenya and Uganda were asked if they could help with personnel. As a result, Mr. (now Dr.) J. D. Gillett and Mr. R. B. Highton were seconded temporarily to assist. It was particularly useful to have Gillett's help, not only as he was an experienced entomologist, but also because he had worked in Bwamba previously, under Mahaffy.

The general plan was to build platforms at varying heights in the trees at Mongiro and to conduct a series of 24-hour catches simultaneously at all levels, with a ground-level control, as in the stratified catches in banana plantations. With the help of Gillett and Highton and a much augmented African staff this work went ahead rapidly, platforms being built at 16, 31 and 54 feet (the last was in a low part of the main canopy). A nucleus of experienced staff was available from the stratified plantation catches, and the first series of 24-hour catches was begun as soon as the platforms were ready, ten being carried out. While these were in progress it was decided that a second set of platforms should be built in the adjacent forest area of Mamirimiri, just across the deserted clearing of Bamaga. The forest at Mamirimiri has a higher canopy and is less swampy than that at Mongiro. The heights of this second set of platforms were 22, 44, 58 and 82 feet. The highest was in an emergent tree and was thus clear of the main canopy, while the 58-foot platform was in a very dense part of the canopy. Here a comparable series of ten catches was made. The African catchers, at first rather apprehensive about work at the higher levels, soon became accustomed to the new method and worked very well indeed. Yellow fever virus was not obtained, though the number of mosquitoes taken was large, 17,811 in all.

These catches, the first stratified 24-hour catches to be carried out in forest, yielded a considerable amount of new information about mosquito biting behaviour, and now for the first time the complexity of this subject began to be appreciated. Each zone - the canopy, the understorey and ground level, showed its own predominant group of species, and each species showed its characteristic biting-cycle. The results have been reported in detail elsewhere (Haddow, Gillett and Highton, 1947) and here only the main findings will be discussed.

Anopheles gambiae was, rather remarkably, the commonest mosquito. None of the workers concerned had ever met this normally endophilic species in such numbers as in the uninhabited forest blocks of Mongiro and Mamirimiri. Though its main zone of activity was just above the ground, it was the commonest mosquito at every level. Thus, while it could be said that it was essentially a ground-level species, it was necessary to remember that very considerable numbers were taken even in and above the main canopy. A. gambiae, as noted above, cannot transmit yellow fever virus. The biting-cycle was similar to that shown in the first 24-hour catch series, the main wave of activity being in the period before sunrise. This curve closely resembled the entry curve of this mosquito to huts at Kisumu (v.s.).

Several species of Taeniorhynchus occurred in the canopy, but the numbers of this genus taken were small at all levels in this series of catches. Eretmapodites spp. were virtually confined to ground-level and as a result of this finding, interest in this genus (in so far as yellow fever transmission was concerned) waned considerably. Culex spp. were quite common at all levels, but these mosquitoes have never proved of interest in relation to yellow fever, though they are important in the transmission of various other viruses such as, for example, West Nile virus and the virus of Japanese B encephalitis.

Main interest centred naturally on the genus Aëdes, and here considerable differences in vertical distribution were found. In these catches and in another comparable series of 10 catches at Mongiro and 10 at Mamirimiri, which was carried out during the following year, 450 mosquitoes of the subgenera Aëdimorphus and Banksinella were taken and of these 379 (84 per cent) came from the ground-level stations. Only 27 (6 per cent) were taken in or above the canopy - a figure averaging less than one specimen per catch. The only member of these subgenera which showed arboreal tendencies was A. natronius which, though included in the infected lot, was not considered suspect as its distribution is very limited. A. natronius is a brackish-water breeder and is confined to the areas around hot springs, salt and soda lakes, etc. At Mongiro its breeding-grounds are around the Mbuga and Nyansimbi hot springs. It is scarce elsewhere in Bwamba. It was now felt that A. cumminsi and A. argenteopunctatus, both members of the subgenus Aëdimorphus, could probably be eliminated from the list of suspect species. Altogether 49 A. cumminsi were obtained at ground-level. A. argenteopunctatus was not taken in any of these 24-hour catches and its very rarity, combined with the ground-haunting habits of the subgenus to which it belongs, were considered as valid reasons for eliminating it from the list. It may be mentioned that work in later years confirmed that this is a ground-level species.

The other three subgenera encountered, *Mucidus*, *Finlaya* and *Stegomyia*, were all to a large extent arboreal and it is convenient to discuss them together. All were relatively scarce at ground level (with the single exception of *A. dendrophilus* - not many specimens of this mosquito were taken in the catches under discussion, but the result has been confirmed by subsequent work). In the understorey *A. apicoargenteus* was the dominant species, biting by day. In the present series *A. ingrami* was too scarce to show a clear distribution picture, but subsequent work has shown that this also is an understorey species, with a clearly marked peak of biting activity in the hour before sunset. In the canopy there was a most interesting succession of species. *A. longipalpis* was the first to appear, biting mainly in the middle of the day and early afternoon. *A. africanus*, which was now found to be the dominant mosquito of the canopy, was scarce by day. In the hour after sunset it showed an exceedingly well-marked wave of biting activity, after which the numbers taken fell again, though some biting went on during the night. Finally, *A. grahami*, a member of the subgenus *Mucidus*, became active late at night. Only a few *A. grahami* were taken in these catches, but the result was amply confirmed by later work, both for this species and for other members of the subgenus (Haddow, 1954). It may be noted that up till this time members of the subgenus *Mucidus* had been virtually unknown as biting mosquitoes. The reason now became obvious.

It was now considered that *A. africanus* was an exceedingly likely suspect. Thus not only was it the dominant Culicine of the canopy, biting by night, but it was also very regularly present. Thus it was taken in 17 of the 20 catches made in 1944. The numbers taken at different levels were as follows:

Mongiro	54 feet	49
	31 feet	23
	16 feet	14
	Ground level	3
Mamirimiri	82 feet	70
	58 feet	150
	44 feet	76
	22 feet	27
	Ground level	17

The Mamirimiri figures are the more interesting as here it is seen that the numbers increase steadily till the canopy is reached at 58 feet, and thereafter fall again (in the emergent tree, the platform at 82 feet being above the canopy).

An apparent anomaly in the first 24-hour catch series (Haddow, 1945b) was now explained. There it was noted that while A. africanus larvae were prevalent in forest and absent or at least very scarce in plantations, the adults were apparently more often taken biting in plantations than in forest. It happened that most of the plantation catches were made in a plantation on the edge of an exceedingly steep and heavily forested ravine. Thus the crowns of the upper trees were on a level with (and at most only a few yards distant from) the banana plants. Thus A. africanus was apparently merely diffusing out horizontally in this particular case from what was now realised to be its usual habitat.

The number of mosquito species showing an obvious preference for the canopy was not large, and it is to be noted that most species, while showing a preference for one particular level were taken at most or all levels. An unexpected and most interesting finding was that various Tabanidae have arboreal tendencies and that one species, Chrysops centurionis is predominantly an inhabitant of the canopy, biting - like A. africanus - in the hour after sunset. This was the first observation of arboreal activity among the Tabanidae, and it was considered to be of some importance as C. centurionis is very closely allied to the classical vectors of loiasis in West Africa, C. silacea and C. dimidiata. It is interesting to note that since the discovery of arboreal activity in C. centurionis, both these species have been found to be arboreal, and much work has now been done on this subject in relation to human and simian loiasis in the Cameroons, the most important papers on the entomological side probably being that by Crewe and O'Rourke (1951) and those by Duke (1955a, b, c and d).

These twenty catches concluded a somewhat arduous spell of work, continuous from mid-April till mid-July. They were carried out in short series of 3-5 catches made non-stop, and they were under continuous European supervision both by day and by night. Large and extensive catches at ground-level were also included in the programme, while the platforms were being built and also during pauses in the 24-hour catch programme. At the end of the last Mamirimiri catch, work had to be broken off to rest the European and African staff. Though it was a disappointment that yellow fever virus was not obtained, it was felt that work could now proceed on sounder lines, and that eventually there was good hope of success. Mr. Gillett and Mr. Highton now returned to their Departments, having given help of the highest order, often under the most trying conditions, in hot wet forest, at all times of day and night.

When work was taken up again by the writer, it was felt that the vertical stratification of forest mosquitoes might be influenced by micro-climatic differences at different levels, and it was decided that this should be studied. As noted above, only two sets of apparatus were available. Using these, the ground-level station at Mongiro was compared with the platforms in succession. The results were of considerable interest. There was very little difference between conditions at ground-level and those at 16 feet where temperature and humidity were concerned. There was, however, a striking difference between ground-level and 31 feet, which registered conditions not very different from those to be expected in the open air. Between the 31-foot and 54-foot levels there was not much difference. These results closely resemble a series of observations made by Allee (1926) on the microclimate at various levels in the rain-forest of Panama, and this seemed to indicate that this type of climatic stratification might be of rather general occurrence in rain-forest. Its main interest in the present connection was that most of the obviously specialised arboreal mosquitoes had their main distribution above the level at which the largest change was noted - i.e., they were scarce till the 16-foot level was passed. Even though the weather was warm and dry at the time when these readings were taken, the striking equability of the microclimate just above the forest floor was confirmed. Later readings taken at Mamirimiri during the rains showed that in wet weather the differences between the lower and upper levels were greatly reduced, just as in wet weather the microclimatic barrier between the forest edge and the open air also tends to be greatly reduced.

The other main work of 1944 was the collection of a further monkey sample and during the year 62 were obtained, including two infants (one positive). In the remainder of the sample the overall immune rate was 28/61, or 46 per cent. By the end of the year the Bwamba total had thus risen to 160 monkeys, 3 of which were infants. The immunity rates were now as follows:

Infant	2/3	-
Juvenile	0/24	(0%)
Subadult	6/24	(25%)
Adult	66/86	(77%)
Old	23/23	(100%)

The overall immunity rate in the entire sample (excluding the infants) was thus exceedingly high, being approximately 61 per cent.

When the sample had reached a total of 150 monkeys it was considered adequate for detailed analysis, and the results were written up (Haddow, Smithburn, Mahaffy and Bugher, 1947). Many of the conclusions set out in this paper have been discussed above, and for detail the paper itself should be consulted. Additional points brought out were as follows:

In analysing immune rates by habitat, sex and species, it was found important to reduce the crude data of the various groups to a standard age-distribution in which the frequencies in the various age-grades were made proportional to those in the total sample. No significant differences were found in the incidence of immunity as between species or sexes. With reference to locality, the Ruwenzori foothills remained an area with no immune monkeys, while in the lowlands the immunity rate was very high. The incidence of immunity increased with increasing age, as shown above and in this connection it was found that on the hypothesis that all age-grades were equally exposed to infection a theoretical curve could be constructed which fitted the observed data very closely indeed. It was concluded that in Bwamba the non-immune section of the monkey population was being immunised at the rate of 27 per cent per year. When the distribution maps and what was then known of habits and habitat were considered in relation to the distribution of immunity in man, it was concluded that the redtail monkey, Cercopithecus ascanius schmidti, a forest-edge species, notorious for its plantation raiding activities, was probably the species mainly responsible for bringing the virus into the immediate vicinity of human settlements, while the lowland colobus, Colobus abyssinicus ituricus, was probably the main species involved in the monkey-to-monkey cycle of the uninhabited forest. This monkey does not raid plantations, and very rarely descends to ground level. It is probably the commonest species in the high-canopy areas and the forest interior.

In this first monkey paper the nomenclature given by G. M. Allen in his "Check List of African Mammals" (1939) was followed. At a later date it was concluded that many of the names he lists cannot be accepted - it is well known that the nomenclature of monkeys is both chaotic and controversial. Consequently, some of the names now in use at the Institute differ from those in the paper under discussion. The two species mentioned above, for example, appear there as Cercopithecus nictitans mpangae and Colobus polykomos uellensis. Much of the trouble arose originally from the fantastic multiplication of species and subspecies - usually on entirely inadequate and poorly documented material - which occurred around the turn of the century, the school of systematists involved being led by Matschie of the Berlin Museum. It would be hard to name anyone who has done greater disservice than Matschie to the systematic side of mammal work in Africa.

To sum up the main results for 1944: It was finally established, by a virus isolation, that yellow fever occurs in the rain-forest, in the absence of man, the vector being neither A. aegypti nor A. simpsoni.

Work in the forest showed that different mosquitoes prefer different levels, the dominant species of the canopy being A. africanus. Both on practical and on theoretical grounds there was every reason to suspect that this mosquito was the vector in the monkey-to-monkey cycle of the forest. A minor, but interesting finding was that in banana plantations monkeys are liable to be bitten by A. simpsoni even in the crowns of the largest plants. Further sampling gave additional information about the role of the local monkeys as mammalian hosts of the virus.

Work carried out during 1945

At the beginning of 1945 there was a spell of exceptionally dry weather in Bwamba, and it was felt that there should be a repetition, under these changed conditions, of the previous year's tree-platform work, which had been carried out during the rains. It was considered that the new series should be comparable with the old and that, as before, there should be ten 24-hour catches at Mongiro and ten at Mamirimiri. It was already known that most species would be reduced in numbers, but it was not known how the vertical distribution might be affected by the dry weather. A still more important point was to establish whether or not the various Stegomyia spp. - and particularly A. africanus - could still be taken as adults in the trees, or whether some of them might depend entirely on their drought-resistant eggs (characteristic of this subgenus) to carry them through dry periods of this kind. It will be understood that for the effective transmission of a disease where the agent does not persist in the blood of the mammalian host for more than a few days, the mosquito vector, which is the only known reservoir, must be present constantly as an adult.

These new series which were carried out by the writer in February and March, using the same platforms and catching teams as before, showed that in most of the mosquito species there was a tendency to concentrate in greater proportion in the zone which had been preferred under wet season conditions. Similarly, there was a tendency for biting to be concentrated at the optimum hours, the scatter round these being less than that noted in wet weather. Generally speaking, however, the differences in behaviour were much less in degree than had been expected and were often ill-defined. A. africanus was taken in the tree-tops in both areas, and thus could be assumed to inhabit this environment as an adult at all seasons.

An interesting finding concerned A. gambiae, as here the numbers were only slightly reduced. The reason was that the main local breeding-grounds are the footprints of game animals around swamps which never dry out (though their edges may advance or retreat) as they are fed by hill-foot seepages and springs and by small streams which flow all year round. In these dry-season catches 14,864 mosquitoes and Tabanids were taken and 14, 084 (95 per cent) of these were A. gambiae. When the whole series of wet season and dry season catches is combined, it is found that the total yield was 32,675 mosquitoes and Tabanids, of which 30,240 (93 per cent) were A. gambiae. This is a striking demonstration of its prevalence in the uninhabited forest at Mongiro and Mamirimiri. It may be noted that some years later Lumsden (1952), working on the same Mongiro platforms, concluded that there was a marked reduction in the numbers of this mosquito during dry weather. This result, however, appears to be the result of a fallacy, as Lumsden based his argument on catches made during the sunset period, and not on 24-hour catches. In the first place, the sunset period does not sample the main wave of activity of A. gambiae, which occurs shortly before sunrise. In the second place the present writer (1954) has shown that in dry weather there is a tendency for A. gambiae to begin biting later. This is compensated by a relatively greater wave of activity later in the night. Thus Lumsden was selectively sampling the period most likely to be misleading. Had he based his views on 24-hour catches or on all-night catches his conclusions would, it is believed, have been very different.

In the dry weather the arboreal Tabanid C. centurionis showed a marked alteration in habits. While in the wet season catches at Mamirimiri the preferred levels were in the canopy and above it, the dry-season catches showed the main concentration well below this, in the zone sampled by the 22-foot platform. At Mongiro the wet season catches produced only one C. centurionis and are therefore valueless for comparative purposes. In the dry weather, however, a fair number were taken, and these occurred mainly at 31 feet - in other words, in the same stratum as at Mamirimiri. Thus in hot dry weather in Bwamba this Tabanid appears to be driven down from the canopy to a zone 20-30 feet above ground, and current work in the Entebbe area suggests that where the canopy is lower than at Mongiro, it may be driven down further still.

At the conclusion of these catches the mosquito work on platforms was written up (Haddow, Gillett and Highton, 1947). The work on C. centurionis was held over, pending further investigations later in the year.

It was felt that now the circumstantial evidence incriminating A. africanus was sufficiently strong to justify the entire routine field work of the year being concentrated on this species, and it was felt that a major effort must be made to isolate virus from it. A plan was therefore drawn up for three months' intensive catching, during the rains, from April till June. The catches were to be made in the evenings (in practice they covered the period from 1600 or 1700 hours till 2030 or 2100 hours, Local Mean Time) and all the Mongiro and Mamirimiri platforms were to be used. Mosquitoes were to be sent to Entebbe for inoculation, only four groups being retained. These were A. africanus, A. aegypti, "Other Aëdes" and Taeniorhynchus spp. When, however, catching was actually begun, it was found that the transport difficulties were too great to allow the work to proceed smoothly, and it was decided that all the inoculations would have to be carried out in Bwamba. The small field laboratory was therefore rapidly adapted and mosquito-proofed, and rhesus monkeys and mice were brought out from Entebbe. In the case of A. africanus six monkeys were used in daily rotation. Other groups were inoculated twice weekly. All the A. africanus which reached the laboratory in good condition were given the opportunity of biting a monkey before they were ground up for inoculation.

Almost as soon as the work was begun, it was found that the yield of A. africanus from the two low platforms at Mongiro was too small to be worth while. The 16-foot platform was therefore abandoned, and the

31-foot platform was raised to 51 feet. In addition four new platforms were built at heights of 55-59 feet. Sixty-four catches were made on the high platforms at Mongiro, and 58 on the Mamirimiri platforms (22-82 feet). Various minor series which were carried out for control purposes need not be discussed here. Altogether 8,053 mosquitoes were taken, of which 4,553 were inoculated into the monkeys. The total number of A. africanus taken was 3,593, of which 3,110 were inoculated in the Bwamba laboratory. Of these 2,883 fed on a non-immune rhesus monkey before being ground up for inoculation. Virus was not obtained during this work, but a good deal of information was gained about A. africanus, as follows:

This species was confirmed to be the dominant Culicine mosquito of the forest canopy. Thus in the catches on the six high platforms at Mongiro (all in the main canopy) 3,776 mosquitoes were taken, and of these 2,113 (or 56 per cent) were A. africanus (this percentage would of course have been less had 24-hour catches been involved; the present series covered selectively the most favourable period for A. africanus). At Mamirimiri the sample was less critical, as catching was carried out at heights ranging from 22-82 feet, all mosquitoes being pooled. Here 3,062 mosquitoes were taken, of which 1,463 (48 per cent) were A. africanus. Not only was it the commonest mosquito, but also the most regularly present. It was, in fact, the only Culicine which was taken on every day of the catches. This was considered an important point, as it had begun to be felt that where the transmission and maintenance of disease are involved, regularity of occurrence must be almost as essential as numerical abundance.

Weather had an obvious influence on the biting-activity of A. africanus. On chilly, wet or windy evenings, few were taken (this was later confirmed by Lumsden, 1952, using the same platforms). The highest numbers were taken on warm, quiet evenings after sunny afternoons.

An interesting point arose during the feeding of these mosquitoes on a susceptible rhesus monkey, in the hope of obtaining a direct transmission by bite from wild-caught mosquitoes. The mosquitoes were fed individually, and altogether 3,323 were in good enough condition to be worth trying (a damaged mosquito can seldom be induced to bite). Of these 2,883 (87 per cent) bit the monkey and 2,808 (85 per cent) engorged fully. Considering the reluctance with which most wild-caught Culicines feed on blood in captivity, this is considered to be a strikingly high figure - quite remarkably high in so far as the writer's personal experience is concerned. It suggests that monkeys are a preferred host of A. africanus. Another most interesting point was the unusual speed with which engorgement occurred. It was often complete within 30 seconds of the first insertion of the proboscis. In most catches a certain number of specimens were brought in which had wholly or partly engorged on the mosquito catchers before collection. As these were less likely to feed on the monkey this matter was taken up repeatedly with the catching teams. Finally it emerged that, in comparison with most other species, A. africanus makes a particularly silent and direct approach to its host and that its bite is remarkably painless. Thus its habits are obviously well suited for attack on monkeys in their sleeping trees.

A point in taxonomy which arose during this work was that intergrades were found between A. africanus and the closely allied A. luteocephalus. As it had already been found that (in Bwamba at least) the characters used in distinguishing the larvae were not reliable, it was now suggested that full specific distinction between these mosquitoes was not justified.

During these catches two A. aegypti were taken at Mamirimiri. As the catches from all levels were pooled, it was not possible to tell at what height these had been taken. At Mongiro, however, 13 were taken actually in the canopy of the uninhabited forest. This was a most interesting finding, strongly supporting the belief that in Bwamba A. aegypti, usually the most "domestic" of all mosquitoes, was still to be found in its primitive wild state.

For details of the mosquito work the paper on these catches (Haddow and Mahaffy, 1949) should be consulted.

Chrysops centurionis was very prevalent at Mongiro and Mamirimiri in the period concerned, 706 being taken. There was now a good deal of information about its biting habits and vertical distribution, but the work was not written up till a good deal later (Haddow, Gillett, Mahaffy and Highton, 1950). In this paper (which it is convenient to summarise at this point), it was pointed out that the biting-cycles of C. centurionis and A. africanus are strikingly similar, and it was suggested that light may be of importance in relation to the crepuscular peaks of biting activity shown by these insects, as it is the only factor which changes with sufficient rapidity at that time to explain the sudden onset and short duration of this activity. It is to be remembered that near the equator darkness falls with striking rapidity after the actual moment of sunset. It was also suggested that monkeys are the most likely host for this Tabanid. It was pointed out that it is very closely allied to the known vectors of human loiasis, and the view was advanced that it might be a vector of the filarial infections so common in monkeys. This has recently been confirmed in West Africa by Dr. B. O. L. Duke (private communication). It was also pointed out that other species of Tabanids had also been taken in trees in Bwamba, and that there was evidence of nocturnal activity in the case of six Uganda species. It was suggested that the enormous size of the eyes of some Tabanids (often with striking colour patterns and facets of more than one size) might have a relation to nocturnal activity.

While analysing the figures for this Tabanid the writer had been struck by the need for some index figure which would indicate not only the numbers of insects taken at a given hour in a series of catches, but also the regularity of occurrence at this hour. The first idea was to multiply the mean catch for a given hour by the number of days on which the insect concerned was taken at that time. It had, however, been pointed out by Williams (1937) that the arithmetic mean is seldom a sound measure in the case of a series of catches of insects, and that a modified form of the geometric mean is to be preferred. Therefore, instead of the arithmetic mean, the geometric mean multiplied by the number of days of occurrence was taken as the "biting index". This figure, worked out to two decimal places, was usually multiplied by 100 to give integer numbers.

At a later date the writer abandoned the use of this index as he considered that, the geometric mean itself taking some account of the consistency of the observations, the index was weighted too much in favour of days' occurrence as opposed to numbers taken. This matter has been discussed more fully elsewhere (Haddow, 1954).

It may be mentioned that between the time when arboreal activity was discovered in C. centurionis (1944) and the present, very considerable numbers have been inoculated into laboratory animals. So far, however, no virus has been isolated from this species.

The intensive catching programme of 1945 was very expensive, as there was a good deal of local travel and much extra staff. It was also exceedingly exhausting. Mongiro is 15 miles from the field station at Bundibugyo, and much time was spent in travelling between these points. Most of each morning was spent on feeding the previous night's catch of A. africanus on a monkey and in making the inoculations. The early afternoon was given up to notes and records. About mid-afternoon the teams were taken down by car to the platforms. They did not get back to Bundibugyo till about 9.30 p.m., and sorting and identification of the catch was rarely finished much before midnight. Though a good deal of information was gained, virus was not obtained, and it was decided that in future intensive efforts to isolate virus in forest would not normally be made at the expense of other work unless there was evidence of virus activity in a given area. It was considered that the best way to attack the problem would be to establish "sentinel" rhesus monkeys on platforms in the canopy right along the edge of the main forest from Mongiro to Hakitengya, near Bundibugyo. At Hakitengya a new house and laboratory were built, as the old station was not really adequate for work involving inoculations and attempts to isolate virus actually on the spot. Rhesus monkeys were now installed on five of the Mongiro platforms, on the canopy platform at Mamirimiri and on a chain of 11 new platforms extending from Mamirimiri to Hakitengya. The monkeys were in roofed wooden cages with expanded metal front and bottom. They were fed each morning and at the same time their temperatures were taken, three permanent camps of monkey watchers being established for this purpose. Once per month a blood sample was taken from each for yellow fever protection test at Entebbe. The hope was that a sentinel would become infected and, as the incubation period is short and the disease usually fatal in the rhesus monkey, that work could be concentrated in the infected area without loss of time. It had become evident, from work in Africa and South America that the virus does not remain in a given small area for more than a short time. No results of interest came from the sentinel programme during 1945.

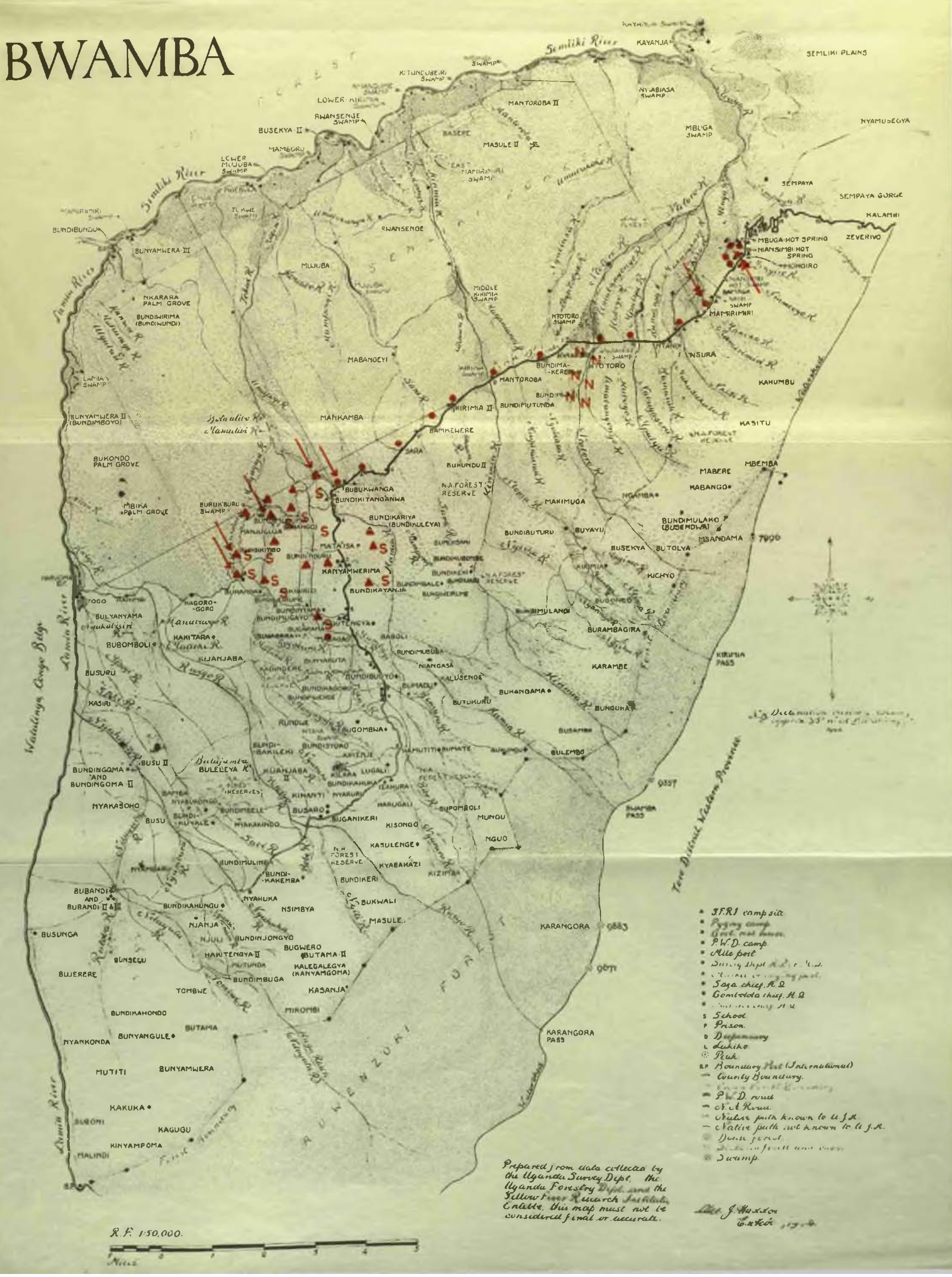
During the year the collection of wild monkeys went on, 48 being obtained, of which 23 (48 per cent) were immune to yellow fever. There were also six infant monkeys, two of which were immune. The total Bwamba sample now stood as follows:

Infant	4/9	-
Juvenile	0/24	(0%)
Subadult	6/35	(17%)
Adult	78/108	(72%)
Old	34/38	(89%)

The overall picture was thus the same, with a general immunity rate (excluding infants) of 122/205, or 59 per cent. On the other hand, the immune rates in the 1945 sample had been lower than usual. For example, there were no immunes among the 11 subadult monkeys collected during the year. This was taken as possibly indicating a lowered activity by the virus.

In summing up the results of the year's work where yellow fever is concerned, it may be stated that they were disappointing. It was, however, firmly established that A. africanus was the most likely vector. In addition, a change in method - the use of sentinel monkeys on a fairly large scale - was introduced. An equally important change in method was permitted by the construction of the new laboratory at Hakitengya, as now all the preliminary processes could be carried out on the spot, up to the primary isolation of virus and the first passage in experimental animals.

Fig. 5: The disposition of sentinel monkeys in Bwamba. The original forest sentinel stations, established in 1945, are shown as red circles. Those set up in 1946 are shown as triangles. The stations in banana plantations, established in 1946, are shown by the letter S. The forest sentinels established in the Ntotoro Valley in 1947 are shown by the letter N. Arrows indicate the stations where sentinels became infected in 1948 and the station where infected Phlebotomus spp. were taken. Mosquitoes from various stations were usually pooled and it was not possible to tell from which particular platform the infected specimens had come. Double-headed arrows show the stations from which came the infected mosquitoes taken in 1947.



Work carried out during 1946

Early in 1946 Dr. G. W. A. Dick, who had recently joined the team, carried out a survey of immunity among children in Bwamba. Every year since the mass vaccination of 1941, Mahaffy had tested a large sample of the people, to make sure that the immunity brought about by vaccination was holding good, and his sampling, which continued till 1945, showed that almost 90 per cent of the population were still immune at that time, and that there was thus no danger of an epidemic (Dick and Smithburn, 1949). It was considered important, however, to find whether children born after the vaccination campaign were being infected with the virus. The new results (Dick, 1950) showed that not only was this occurring, but that in some forest edge localities, more particularly in the original study area described above, the incidence was extraordinarily high, the great majority of children having become immune by the age of four. As there was little possibility of man-to-man spread among a population where the immune rate due to vaccination was so high, it was concluded that virus must be reintroduced from the forest at frequent intervals.

As the occurrence of immunity in young children in the original study area indicated very recent activity by the virus, and also as the immune rates there were particularly high, it was decided that an effort should be made to achieve an isolation within this part of the lowlands, as it was considered that work in the main forest area should depend on the results of the sentinel monkey programme. The first thing done was to instal sentinel monkeys in trees throughout the greater part of this area. These covered the whole of the stretch of country now under study, whose limits were the forest boundary to the north, the Humia valley to the east, and the Hakitengya-Buhanda footpath to the south-west. This area was later known as the "Hakitengya triangle". There were 16 of these new tree platforms, and in addition 12 sentinel monkeys were installed near ground level in banana plantations within the triangle. There were now 45 sentinel monkeys in Bwamba (Fig. 5) and they were cared for and observed by seven separate camps of monkey watchers. Temperatures were taken daily, and once per month each monkey was brought in to the main laboratory and bled from a leg vein for protection test.

It was also decided that a short, intensive effort should be made to isolate virus from mosquitoes caught in banana plantations, as it was obviously in this habitat that small children - who do not wander in the forest - were acquiring their immunity. The twelve plantations in which sentinels had been installed were chosen for this purpose, and the catches were arranged so that a collection was made in each once per fortnight. The work went on till each plantation had been sampled four times. Altogether 37,026 mosquitoes were taken, but virus was not isolated, and none of the sentinel monkeys in the plantations became infected. Though the work was unproductive from the virus point of view, it yielded results which were interesting in other respects, as follows:

It had been difficult, when stationing the sentinel monkeys, to find really suitable plantations. The cultivation of "dry" or "hill" rice was at that time increasing rapidly in Bwamba, at the expense of the banana plantations, and the latter were jungly and unweeded. The result was that, instead of the short species list usually associated with plantation catches, 42 species and groups of mosquitoes (including 23 species and groups of Aëdes) were taken. Further, A. simpsoni was no longer the commonest mosquito, being outnumbered by A. circumluteolus. The more interesting and abundant species were as follows:

A. aegypti	34
A. simpsoni	7,334 (20%)
A. africanus	7
A. cumminsi	5,017 (14%)
A. circumluteolus	17,565 (47%)
A. palpalis	1,926 (5%)
Other species	5,143

Generally speaking, the prevalence of A. simpsoni was found to be inversely proportional to the prevalence of the other species. It was quite common in the few well-tended plantations, but definitely scarce in those which were weedy and overgrown. In the least favourable plantation of the twelve, it made up only six per cent of the total catch.

When these catches were completed, no further mosquito work was carried out in the plantations, but the sentinel monkeys were left in situ.

Shortly after the end of the series, however, some mass-catching in forest became necessary. The routine monthly test on the tree sentinel monkey at Hakitengya gave an inconclusive result, and this was duplicated on retest. It was established later that this monkey was not, in fact, immune, but at the time it was necessary to assume that it had undergone a mild, subclinical attack of yellow fever, and was in process of becoming immunised. The various mosquito catching teams were at once concentrated in this single area and a mass catch on a very large scale was made. There were four ordinary day catches, each made by four teams, the ground covered around the sentinel tree being about ½ square mile. There were also six catches on the sentinel platform, each covering the period 13-21 hours, Local Mean Time. Finally, two mass 24-hour catches were made, in which relays of catchers worked throughout the day in the forest and continued throughout the night with the aid of lamps. The yield from this catch was very high, 14,657 mosquitoes, belonging to 49 species and groups, being taken in six days. No virus was isolated, but the incident was a useful test of preparedness.

Altogether, a rather intensive effort to isolate virus was made during 1946. Not all the mosquitoes discussed above were inoculated into animals, several groups being discarded. On the other hand, mosquitoes from the investigative catches discussed below were often used in this way, and this was always done in the case of A. africanus. Altogether, 45,924 Bwamba mosquitoes were inoculated into mice and monkeys during the year, without a single virus isolation, and none of the 45 sentinel monkeys became immune.

Yellow fever studies in many areas, such as Brazil and the Belgian Congo, are guided by the use of the viscerotome (Rickard, 1931), as the lesions in the liver in yellow fever are pathognomonic. The viscerotome is an instrument by the aid of which relatively unskilled personnel can obtain liver specimens, without full autopsy, for histological examination, and usually a specimen is taken from any person dying from an undiagnosed fever of less than ten days' duration. Routine viscerotomy had been carried on in Bwamba during Mahaffy's first work, in the hope of localising areas of virus activity, and it was now reintroduced. It was carried on throughout 1946, but had to be abandoned early in 1947, owing to intense local opposition (it was supposed that the specimens were being used by the Institute for witchcraft) without any positive specimens having been obtained.

When the main sentinel monkey programme was begun, it was hoped that it would soon prove productive. As time went on, however, without any positive result, it was felt that, while A. africanus was known to feed readily on monkeys in captivity, there was no final proof that it did so in the canopy. As trap-catching was considered to be unlikely to produce the type of results required, it was difficult at first to know how to proceed. It was, however, felt that if a catcher sat beside a monkey bait and collected the mosquitoes which alighted to bite the animal, this would at least show which mosquitoes would bite a monkey even in the presence of man, and thus also in the presence of a much larger bait. It was felt that if reasonably large numbers of A. africanus bit the monkey under such conditions there could be no further doubt as to their liking for this type of host. It was obviously necessary that the monkey should be anaesthetised or narcotised, and further, as only a few monkeys of a given species could be made available at a time, that the anaesthetic should have a prolonged action. Dick, after a number of trials, produced satisfactory and prolonged anaesthesia by injections of 'Nembutal' (sodium ethylmethylbutylbarbiturate). Monkeys would remain under deep anaesthesia after the injection for periods up to twelve hours. Body temperature tended to drop rapidly, but was easily maintained by laying the animal on a hot water bottle wrapped in a towel. Using this method, three 24-hour catches were now carried out with monkeys as bait.

In the first catch three very small redtail monkeys (Cercopithecus ascanius schmidti) were used in rotation, the catch being made on the canopy platform at Mamirimiri (now altered to 57 feet). This monkey is a common species of the forest edge, and a consistent raider of plantations. The weather was most unfavourable, a cool cloudy morning and a rainy after-noon and evening being followed by a chilly night. In weather of this kind most mosquitoes bite with reluctance. Nevertheless, 12 mosquitoes and 1 Tabanid were taken on the bait, the details being as follows:

A. gambiae	1
A. longipalpis	2
A. apicoargenteus	1
A. africanus	8
Chrysops centurionis	1
Total	13

During this catch only 3 A. africanus alighted on the catcher, in spite of the fact that he represented a very much larger bait than did the monkey. Another interesting point was that while A. gambiae attacked the catcher continuously throughout the night, only one alighted on the monkey.

In the second catch, carried out on the same platform, weather was better and the yield higher. Grey monkeys (Cercopithecus aethiops centralis) were used. This monkey is another notorious plantation raider. Two monkeys were available and were used in rotation. The result was as follows:

T. africanus	8
A. grahami	2
A. longipalpis	3
A. ingrami	1
A. apicoargenteus	5
A. africanus	23
Culex sp. indet.	1
Chrysops centurionis	16
Total	59

It will at once be noted that A. africanus made up almost half the catch and that C. centurionis was also very common. As noted above, C. centurionis had been considered, on theoretical grounds, to be a probable vector of monkey filariasis (Haddow, Gillett, Mahaffy and Highton, 1950), and the above result lent support to this view.

It may be mentioned that by this time there was a good deal of work going on in other parts of Uganda, between spells in Bwamba. One part of this other programme had entailed the building of tree stations in forest at Zika and Kitinda near Entebbe, each station consisting of a platform in the canopy and one in the understorey. Series of catches were carried out on these platforms, using both levels simultaneously, with a control at ground level (this work will be discussed further below). It was now decided that, as the monkeys used as sentinels were all rhesus, a catch should be made using rhesus as bait, to confirm in their case the results obtained with African monkeys. It was more convenient to carry out this work at Entebbe, and a catch was made at Zika, anaesthetised rhesus monkeys being exposed simultaneously at 52 feet (in the canopy), at 34 feet (in the understorey), and at ground level. The main results were as follows:

	Ground level	34 feet	52 feet	Total
A. africanus	0	14	18	32
Other species	18	25	19	62
Totals	18	39	37	94

Thus though no A. africanus were taken at ground level, this species formed 34 per cent of the combined total for all levels. In the understorey it made up 36 per cent of the catch, and in the canopy, 49 per cent.

It was felt that there could no longer be any doubt that monkeys were to be considered a favourite host of A. africanus. At Zika, for example, it had made up 34 per cent of the catch on monkeys, while in similar catches with human bait it made up only 7 per cent. Further, many series of catches with human bait had by now been carried out in the canopy in various Bwamba localities (see below). When the results of these catches were combined with the results of similar canopy catches at Zika, it was found that in the canopy A. africanus had made up 19 per cent of the total yield. In the three catches with monkeys as bait, however, it made up 45 per cent of the catch from this level. Fuller details of this work have been published elsewhere (Haddow and Dick, 1948).

The construction of these numerous sentinel platforms in Bwamba gave an excellent opportunity for the more detailed study of the mosquito and Tabanid fauna of the canopy. They were distributed over more than twenty miles of forest and of agricultural country interspersed with forest. They were deliberately placed in as many kinds of environment as possible -

climax ironwood forest, swamp-forest with and without palms, second-growth, high mixed forest, low-canopy riverside scrub, gallery extensions, relict patches and so on. In 1947, as will be described below, the sentinel programme was extended still further, the final number of tree platforms being 40. All, however, had one thing in common. No matter in what type of forest they were placed, the platforms were always in the canopy.

Work on these platforms extended over about three years. Six 24-hour catches were made on each, with a control at ground level. While the catches were designed primarily to investigate the mosquito fauna of the canopy, they also provided substantial numbers of A. africanus for inoculation into animals. The work was known as the Bwamba Tree Survey, and it is convenient to discuss it as a whole at this stage. The 240 catches yielded a total of 40,976 mosquitoes and Tabanids, belonging to 85 species and groups. The working out of this sample is still far from complete, and it is not anticipated that it will be finished for some time to come. Only a few small sections of the results have been published. Much of what follows is, therefore, in the nature of a general commentary on the sample as a whole.

Possibly the least expected finding was that, while there were considerable quantitative differences between the catches at different stations, qualitatively they differed relatively little. Thus, while the different species varied in absolute and in relative abundance from station to station, the species list remained substantially the same in forest of all kinds. This has subsequently proved true of areas outside Bwamba also.

The next main finding was that the fauna of the canopy remained much the same, regardless of the height of the platform. Thus in some low-canopy areas of scrubby forest and second-growth the platforms might be only 30-40 feet above ground, while in maturer forest they were most commonly placed at 50-80 feet. The same species were, however, common to almost all of them.

Broadly speaking, the main mosquito groups showed the same trends in vertical distribution as in the original stratified catches at Mongiro and Mamirimiri, the main results being as follows:

In the genus Anopheles there were no species more abundant in the trees than at ground level. The details were:

	Canopy	Ground level
A. gambiae	1,976 (12%)	14,745 (88%)
15 other Anopheles spp.	129 (11%)	1,058 (89%)

Eretmapodites spp. were completely confined to ground level. Altogether 450 specimens, belonging to 5 species and groups were taken at this level, and not one was caught in the canopy. The same was true of the tsetse flies (Glossina spp.), of which 43 were taken, all at ground level.

In the genus Culex the main mass of the catch was made up of unidentified (in fact, unidentifiable) females, but there were also 6 identifiable species, of which only one, C. poicilipes, was more abundant in the trees than at ground level. It may be noted that the arboreal tendencies of this species have been confirmed by work carried out in West Nile District (Lumsden and Buxton, 1951). Altogether 3,239 Culex spp. were taken in the Bwamba Tree Survey, of which 2,539 (78 per cent) occurred at ground level, and only 700 (22 per cent) in the canopy. A similar distribution was noted in the minor genera (Uranotaenia, Ficalbia, etc.) where 20 were taken at ground level and 9 in the trees.

There remain the two major genera Taeniorhynchus and Aëdes, and in these there was considerable diversity of behaviour, some members being arboreal and others preferring ground level. To a large extent the preference went by subgenera, which seems to indicate that these groups are sound biological units as well as being distinct taxonomically.

In the genus Taeniorhynchus the subgenus Coquillettidia, represented by T. metallicus, T. pseudoconopas, T. maculipennis, T. fuscopennatus, T. aurites, T. aureus and T. microannulatus was mainly arboreal. Of the 529 specimens obtained, 365 (69 per cent) were taken in the canopy and only 164 (31 per cent) at ground level. T. fuscopennatus is the only member of this group which is generally most prevalent at ground level, though it is also a common canopy mosquito, as shown by work elsewhere. On the other hand, this series of catches showed why species such as

T. aurites had been inadequately sampled in the past. This mosquito had been recorded from much of tropical Africa, but nowhere seemed very common. Work in Bwamba and at Entebbe showed that as it is mainly nocturnal and arboreal, its prevalence can be gauged only after night work in the canopy. This also applied to some of the other golden species.

In the other subgenus, Mansonioides, the greater numbers were taken at ground level, as follows:

	Canopy	Ground level
T. africanus	1,467 (33%)	3,023 (67%)
T. uniformis	725 (47%)	815 (53%)

Obviously in this sample there is no significant difference between the two levels in the case of T. uniformis, but it is known from work carried out elsewhere by the writer that it is usually much commoner at ground level than in the trees. T. africanus, on the other hand, was obviously more prevalent at ground level in the catches of the Bwamba Tree Survey, but this must not obscure the fact that it was also a common mosquito in the canopy, in fact, the second most prevalent species in this environment. It was, moreover, regularly present, being taken in 206 of the 240 catches, and it occurred in the canopy at 39 of the 40 stations. When it is remembered that it is capable of transmitting yellow fever virus under laboratory conditions (Bauer, 1928) it will be realised that much interest attached to this species. Up till the present, however, it has not been incriminated as a natural vector of yellow fever or of any other virus infection.

In the genus Aëdes 3 subgenera - Aëdimorphus, Banksinella and Dunnius - were found to be made up of ground-haunting species. The results for these subgenera were as follows:

	Canopy	Ground level
Aëdimorphus (11 spp.)	58 (2%)	3,753 (98%)
Banksinella (4 spp.)	34 (1%)	3,365 (99%)
Dunnius (1 sp.)	0 (-)	36 (100%)

All the other subgenera showed at least a predominance of arboreal species. Thus there were three members of the subgenus Mucidus in the present sample.

Altogether 1,008 specimens (94 per cent) of these mosquitoes were taken in the canopy, and only 64 (6 per cent) at ground level. The same was the case with the subgenus Diceromyia. This subgenus is poorly represented in Bwamba, but the results have subsequently been confirmed elsewhere, notably by the work of Bailey (1947) at Gede on the Kenya coast. In the Bwamba tree survey 29 specimens belonging to this subgenus were taken in the canopy, and none at ground level.

In the subgenus Finlaya one species, A. longipalpis, showed a most distinct preference for the canopy, 859 (94 per cent) being taken at this level, as opposed to 57 (6 per cent) at ground level. The other species, A. ingrami, was more prevalent at ground level, where 155 (70 per cent) were taken, as opposed to 68 (30 per cent) in the trees. It is known, however, from subsequent work at Entebbe, that this is actually an arboreal species, but that its preferred stratum is the understorey, which was not sampled in the catches under discussion as all platforms were in the canopy.

The results for the important subgenus Stegomyia will be given by species, as follows:

	Canopy	Ground level
A. aegypti	1	1
A. simpsoni	42 (47%)	47 (53%)
A. apicoargenteus	374 (92%)	33 (8%)
A. fraseri	6	11
A. dendrophilus	25 (24%)	80 (76%)
A. africanus	2,178 (92%)	195 (8%)
A. luteocephalus	7	0
Totals	2,633 (88%)	367 (12%)

In this subgenus A.dendrophilus stands out as the only species definitely showing a preference for activity at ground level. The sample of A. fraseri is too small to permit conclusions to be drawn, but it may be noted that Garnham et al. (1946) have recorded it as common at 55 feet above ground in the Kaimosi Forest in Kenya, the numbers taken being higher than those obtained at ground level. A. apicoargenteus showed a clear preference for the canopy, but it is to be remembered that this is really a mosquito of the understorey, and that therefore the sample under discussion gives a poor estimate of its real prevalence. A. africanus was, as usual, the commonest mosquito of the canopy. It was the only species taken in the canopy at every one of the 40 stations. It occurred at this level in 206 of the 240 catches, a record equalled only by that of T. africanus. The few specimens of A. luteocephalus, which is very closely related to A. africanus, all came from the canopy.

One of the most interesting findings was that A. simpsoni may occur in the canopy. The number taken in the whole series was small, only 89 being obtained, in spite of the fact that many of the forest edge stations were in close proximity to banana plantations. Most of the A. simpsoni did actually come from such stations, but the occurrence in forest was at the best capricious. The fact that almost half of the specimens obtained came from the canopy was a considerable surprise. A. simpsoni was taken in 18 of the 40 localities, and in 13 of these specimens were obtained in the canopy, at heights ranging from 36 to 77 feet. The small numbers, of course, indicate that this species could not be of importance as a vector, even in the extreme edge of the forest - the yield from both levels combined being just over 3 A. simpsoni per 1,000 man-hours. None the less, the fact that this mosquito will enter the canopy indicates another way in which yellow fever virus could occasionally find its way from the monkey population of the forest to the human population of the adjoining banana plantations. This may be deduced from the fact that in East Africa A. simpsoni rarely breeds in tree-holes, and that therefore specimens taken biting in the canopy - where of course they may encounter an infected monkey - will subsequently return to the plantations to oviposit. These observations have been discussed more fully in a separate communication (Haddow, 1950) to which reference should be made for detail.

Turning once more to the genus Aëdes as a whole, it is interesting to contrast the vertical distribution of the mainly arboreal subgenera Mucidus, Finlaya, Stegomyia and Diceromyia with that of the subgenera made up of mosquitoes whose usual haunt is the forest floor, namely, Aëdimorphus, Banksinella and Dunnius, as follows:

	Canopy	Ground level
Arboreal subgenera	4,597 (88%)	643 (12%)
Ground level subgenera	58 (2%)	3,753 (98%)

Little is as yet known of the vertical distribution of the other subgenera of Aëdes occurring in the Ethiopian subregion (Ochlerotatus and Skusea) though Lumsden (1955) has reported on a series of A. (O.) fryeri and A. (S.) pembaensis collected at Gede on the Kenya Coast. The work he carried out included 24-hour catches on platforms, with controls at ground level, and both of these species appeared to be much more prevalent at ground level than in the trees.

An interesting contrast occurred in the case of the Tabanidae. Here 4 species of Haematopota occurred in the sample, and all were plainly more prevalent at ground level, where 42 were taken, as opposed to only 2 in the canopy. In the case of Chrysops centurionis, however, not one was taken at ground level, while 218 were caught in the canopy. The large sample of this species now available permitted a much more detailed analysis of its biting cycle, and also allowed some deductions to be made concerning its relationship to the distribution and epidemiology of loiasis. As, however, it was no longer considered likely to be a virus vector, these subjects are discussed separately in Appendix 6.

The other main work of 1946 was the collection of monkeys, and this showed a very similar result to that obtained during 1945. Of 53 collected by the writer in Bwamba, 21 (40 per cent) were immune, and all of 11 subadults in the sample were none-immune. Thus yellow fever did not show much activity in the local monkey population. During the year it was decided that a sample of mountain monkeys was desirable. A few, mainly juveniles, had been bought from Bakonjo natives. All of these had been found non-immune, but all belonged to age-grades in which immune specimens are in any case very scarce. In view of the fact that human yellow fever was obviously absent or at least very rare in the mountains, it seemed important to find whether this was also the case among the monkeys. The journeys which followed were of an exacting nature, the forested hillsides being exceedingly steep, often semi-precipitous in the valleys, and very wet. Nettles formed a large part of the forest undergrowth, and continuous cold weather, rain and mist led to much dissatisfaction among the porters. Monkeys were few and extremely wary. Eventually, however, specimens were obtained of the uncommon Cercopithecus l'hoesti l'hoesti and of the blue monkey, C. mitis stuhlmanni. Later, a series of Colobus angolensis ruwenzorii was obtained, these being the first complete specimens of this monkey ever collected. It had been known previously only from a few incomplete native-cured skins.

When the total mountain sample was now examined, it was found that it included 3 infants, 9 juveniles, 3 subadults, 5 adults and 2 old monkeys. Not one was immune. All the immune Bwamba specimens came from heights under 5,000 feet. It may be mentioned that in the year under consideration Dick began active collection of monkeys in Bwamba and elsewhere and that a good series of mountain monkeys obtained by him in the Ruwenzori foothills in 1947 confirmed the writer's result, as will be shown below. It thus became obvious that the highland and lowland monkeys must be considered separately when immune rates were to be computed. The results in the lowland sample now stood as follows:

Infant	4/ 14
Juvenile	0/ 18 (0%)
Subadult	6/ 43 (14%)
Adult	91/124 (73%)
Old	42/ 46 (91%)

The results of protection tests on mountain monkeys just discussed led naturally to questions concerning the distribution of A. africanus in the foothills. It was felt that, should it be found that this species, like A. simpsoni, reached its highest level at about 5,000 feet, this might explain the absence of yellow fever among monkeys from the higher slopes. Catches were therefore made at various heights among the foothills and it was found that A. africanus disappeared at about 5,000 feet. Above this it was replaced by a hitherto undescribed Stegomyia which extended upward to about 7,000 feet. This observation was later confirmed by work in the Nyamugasani Valley in the extreme south of the main mountain mass of Ruwenzori. In the Nyamugasani Valley A. africanus extended a little higher, reaching about 5,500 feet, perhaps because the valley is much more sheltered than the exposed and windy North Spur.

The most interesting point about the new mosquito was that, like A. africanus, it proved to be almost entirely arboreal and crepuscular. It is probably confined to Ruwenzori, as it does not seem to occur in the mountain forests of Kigezi, only about 80 miles away to the south. In Kigezi, however, it was found that A. bambusae and A. angustus (also members of the subgenus Stegomyia) were arboreal and crepuscular, and the question now arose as to whether each mountain range might have its own arboreal Stegomyia, biting just after sunset. There has been no opportunity so far to settle this point. It may be mentioned that at Kitale in Kenya A. africanus reaches greater altitudes, namely, about 6,000 feet. Here, however, the country consists mainly of sunny plains, very different from the North Spur of Ruwenzori.

The new mosquito was described under the name A. (S.) ruwenzori by Haddow and van Someren (1950). Though obviously allied to A. africanus the new species differed sharply in pattern, and was also much slenderer in build. This suggested that it might well breed in bored bamboo sections, as some of the bamboo breeders (for example, A. angustus) are slenderly built, but a search of 2,300 fluid-containing sections failed to reveal the larvae. Present and past surveys of larval habitats on the mountain included 300 water-containing tree-holes, 8,203 water-containing plant axils and four small mountain streams. One of the tree-holes yielded three small larvae resembling those of A. africanus, but these were dead when received and so confirmation by rearing was not possible. Attempts to obtain eggs from females were not successful. Later, however, Gillett (1951b) managed to induce females to lay and described the larva, pupa and adult male from bred-out material. His work shows that A. ruwenzori occupies an intermediate position between A. africanus and A. bambusae.

Another question on A. africanus concerned its distribution in the lowland forest. Consideration of the sample of monkeys obtained up till that time suggested that the immune rates in the interior of the main forest block might be lower than those among monkeys from the edges. At a later stage, when the final sample was analysed, it was shown that these apparent differences were not statistically significant (Haddow, Dick, Lumsden and Smithburn, 1951), but at the time it was felt that this matter should be investigated and that the distribution of A. africanus within the main forest should be studied. All catches to date had been made within one mile of the forest edge or else on the banks of the Semliki where once again forest-edge conditions prevail. Now, however, a series of catches was made along a line from the Semliki River through the densest part of the forest to the main road. A. africanus was found to be prevalent at each of the four stations chosen and this was taken as indicating that it existed throughout the lowland forest.

To summarise the yellow fever work during 1946, the main advance was a further gain in knowledge of the habits and distribution of the known vector species, A. simpsoni, and of the suspect vector, A. africanus. Sentinel monkeys were established in many new localities, but without result. It was confirmed that yellow fever infections do not seem to occur in the monkeys of Ruwenzori above about 5,000 feet, which is the altitude limit for A. africanus. Work by Dick showed that the infection was still very common in unvaccinated Africans living in the lowlands near the forest.

Work carried out during 1947

During 1947 mass catching of mosquitoes was again undertaken as it was felt that, while awaiting results from the sentinel monkey programme, efforts to obtain yellow fever virus from humans or mosquitoes outside the main forest should be continued. This work was undertaken at the instigation of Smithburn, who was now Director of the Institute. The object was to localise an area of virus activity in which work could be concentrated. It was felt that the results obtained in the original study area in the previous year had not been very satisfactory, as A. simpsoni was obviously much less prevalent there than it had been formerly. The valley of the Ntotoro River seemed more promising. This valley, a few miles from Mongiro, had been filled by unbroken climax ironwood forest till about 1944-45, when extensive clearings were made along the river banks. By 1947 there was a continuous line of banana plantations extending along the banks, with dense forest on either side. Hut groups were scattered through these plantations. The plantations themselves were mainly gonja bananas, the type most favoured by A. simpsoni, with an undergrowth of Colocasia, another favourite plant for breeding. A census of the people in the valley was made, and blood samples were collected from almost the entire population, then about 200 people, this work being carried out by Dick. It was found that there were 38 non-immune people in the valley and these were now kept under close supervision by an African dispenser, who was installed at an "aid post" built in the valley. The immune rates in the monkey sample from Ntotoro were quite high, being as follows:

Infant	-
Juvenile	-
Subadult	0/3
Adult	6/9
Old	5/6
Total	11/18 (61%)

W. H. R. Lumsden, who joined the team in this year, made some interesting studies on the mosquitoes of the valley, carrying out series of 24-hour catches simultaneously in a hut, in the hut clearing, in the surrounding plantation, and at ground level and in the canopy in the contiguous forest. The results which he obtained here and subsequently in another area where he made similar catches, showed how sharply distinct were the mosquito faunas of these different though closely adjacent environments (Lumsden, 1951a).

The writer at this time built a new series of sentinel platforms in the Ntotoro Valley and discontinued the plantation sentinel programme around Hakitengya. There were now 40 sentinels in Bwamba, all in the canopy, and of these 7 were in the Ntotoro Valley (Fig. 5). Series of catches were made on the new platforms, with controls at ground level, these being the first catches in which the presence of A. simpsoni in the forest canopy was observed. Finally, in order to build up the picture of mosquito life in the valley, four mass 24-hour catches (with moving catchers) were made in the forest, and these yielded 5,546 mosquitoes, including 3 A. simpsoni. A continuous 48-hour catch with moving catchers was also made in the belt of plantations, and yielded 2,788 mosquitoes, of which 1,909 (68 per cent) were A. circumluteolus and only 207 (7 per cent) were A. simpsoni. No other single species numbered as many as 100 specimens. Thus, though the Ntotoro plantations were well tended, the position seemed much the same as that found in the Hakitengya triangle in the previous year, A. simpsoni being relatively scarce as a biting adult. Strictly sylvan species were very scarce in the plantations, less than a dozen specimens being taken. Finally, an axil survey was made, covering colocasia, pineapple, gonja, menvu and bitoke bananas, in each case the sample comprising 1,000 water-containing axils. This showed yet again that gonja and colocasia were the really important plants, that pineapple and menvu were of some importance, and that bitoke was of no importance whatever. Six species of larvae were obtained, of which A. simpsoni was overwhelmingly the most numerous.

When the programme was under way in the Ntotoro Valley, steps were taken to improve the new field laboratory at Hakitengya, so that mosquito suspensions could at all times be made up and inoculated into animals in Bwamba. Previously it had only been possible to do this during selected periods of intensive work, but it now became routine procedure, and of the mosquitoes inoculated into animals during the year, 7,013 were processed and inoculated in the Bwamba laboratory. No yellow fever virus isolations were made. Most of these mosquitoes came from the Ntotoro study area and it may be mentioned at this stage that work continued there till the middle of 1948 without positive results being obtained.

At Entebbe at this time experimental work was being carried out by S. F. Kitchen on the ability of certain species to transmit yellow fever virus, the mosquitoes required being collected and sent in from Bwamba. Those not required for the transmission work were made up into lots for inoculation by Dick. From one lot of arboreal Aëdes spp. (47 A. longipalpis, 17 A. ingrami and 1 A. natronius) an agent was isolated which was later named Uganda S virus. Little is as yet known of this virus. Its main interest at the moment is that where complement fixation tests are used there are irregular cross-reactions with yellow fever virus (Kerr, 1952) and this also occurs in tests employing the new haemagglutination inhibition techniques (Dr. Max Theiler, private communication). It is known that human infections occur (Smithburn, 1952a) but the clinical picture is still unknown. The isolation of this virus was described by Dick and Haddow (1952) and reference should be made to this paper for detail.

It will be realised that by mid 1947 the failure of the sentinel monkey problem to produce results was causing serious concern. Considerable numbers of monkey watchers were employed, and the care and feeding of the line of monkeys, stretching over more than 20 miles of country, was a laborious and expensive undertaking. Each monkey had to be visited daily so that its temperature could be recorded and its food renewed, and once per month all had to be brought to Hakitengya to be bled from a leg vein for protection test - a lengthy task. At first regular visits were made by Dick and Kitchen to collect these blood specimens, but at a later date this work was taken over by the writer and Lumsden.

About the middle of the year it was concluded that possibly A. africanus might not be entering the sentinel cages freely, and that this might explain why the sentinels were not becoming infected. The writer therefore carried out a series of catches designed to investigate this point. This work at once revealed that, though the cages were made mainly of expanded metal with a very wide mesh, only the roof, back and ends being of wood, A. africanus would not enter, even when very prevalent on the platform outside. Mosquitoes could be watched flying round the cage and some species entered it freely, but this did not occur in the case of A. africanus. At the end of a series of observations during which the monkey in the cage was watched closely, the matter was settled finally by putting a small boy (an experienced mosquito catcher) inside an empty sentinel cage on a platform where A. africanus was particularly abundant. The observer outside was able to take this species in numbers, but none entered the cage even when the observer left the platform and descended to ground level, leaving the boy in the cage as the only bait available. It is now known, from long series of catches made in the Entebbe area, that A. africanus will at least occasionally enter large cages made entirely of expanded metal. Apparently it was the somewhat enclosed nature of the cages used at the time which discouraged entry. The wooden top, back and sides had been considered necessary as the Semliki forest is exceedingly wet and rhesus monkeys do not thrive unless they can get shelter from the rain.

As soon as this result was obtained work was begun to adapt the platforms so that the monkeys could be confined on them without cages. Finally they were confined by a running chain to a wire extending the length of the platform and so had good freedom of movement. A shelter for the monkey in wet weather was built in the corner of each platform. Some were, however, attacked and killed by the monkey-eating eagle, Stephanoaetus coronatus, and others were killed by local monkeys and baboons, attracted by the supplies of food.

While work outside Bwamba will be discussed mainly below, it is necessary to digress at this point in order to record an event in the Entebbe area. At Zika, near Entebbe, there were, as mentioned above, six stations (each with platforms at two levels). These were easily adapted, but the Bwamba platforms, differently constructed, had to be completely rebuilt - work which was not finished till the following year. Almost at once after the modification of the Zika platforms one of the sentinel monkeys there was found, at the routine monthly bleeding, to have become immune, and it was possible to say that a subclinical, inapparent infection with yellow fever virus must have occurred between 18th and 30th September. Yellow fever, as known in the laboratory, is almost always a fatal infection in the rhesus monkey, and the fact that this sentinel survived and further, that during the period when the infection must have taken place its temperature record showed no evidence of fever, gave an indication that the monkey-adapted virus of the forest canopy might cause a much milder infection in the rhesus than had been anticipated. This was to be confirmed in the following year.

It was obviously necessary to follow up this case and to attempt to isolate virus from mosquitoes taken on and around the platform concerned. Unfortunately, as yellow fever protection tests take almost two weeks to perform, and as the monkey might have acquired its infection at any time during the previous fortnight, it was already late to begin work. Even then it required another two weeks before the writer could bring in a team from Bwamba. Eight days intensive catching followed, both at ground-level and on the platforms of the station concerned. Virus was not isolated, though 2,313 mosquitoes were taken (catches in the Entebbe area do not produce the huge numbers of specimens usual in catches made in the Semliki Valley). It may be mentioned at this point that altogether 12,881 mosquitoes, including 819 A. africanus were taken at Entebbe for inoculation during 1947, but without isolation of yellow fever virus.

Generally speaking, this episode caused a good deal of disappointment. It had been assumed that rhesus monkeys in the forest would on infection either die or at least exhibit a severe illness, and that - as the incubation period and the course of the disease are both very short in these animals - a team of catchers could be brought into action at the spot concerned within about ten to fifteen days of the time when the infected mosquito bit the sentinel. On the other hand it was considered that the sentinel system, as now improved, would sooner or later lead to incrimination of the vector.

This result was nearly obtained in another way at the end of the year. The short series of catches on the Bwamba tree platforms (the Bwamba Tree Survey) were, during this whole period, being carried on whenever time permitted. Altogether, taking the mosquitoes from the routine work, such as that in the Ntotoro Valley, and those obtained in the tree survey, 41,168 specimens were collected in Bwamba during the year. All the A. africanus, 1,140 in number, and 9,440 other mosquitoes were inoculated. There is evidence that the last lot to be inoculated - a batch of A. africanus from Mongiro and Mamirimiri - was infected with yellow fever virus.

A series of 24-hour catches on the Mamirimiri canopy platform (recently repaired and now placed at 62 feet), with controls at ground level, was carried out in December, 2,957 mosquitoes being taken, including 139 A. africanus. Almost simultaneously a similar set of catches was in progress at Mongiro No. II platform (57 feet) also with ground level controls. Altogether 3,792 mosquitoes were taken, including 48 A. africanus and 1 A. luteocephalus which, as it is regarded at the Entebbe Institute as merely a subspecies of A. africanus, was included in the same lot for inoculation. The two lots of A. africanus were pooled and the inoculation of a rhesus monkey was carried out at Hakitengya by the writer. The monkey concerned had not been subjected to any previous experimental procedure, except that it had been bled for test on December 3rd, the specimen showing that it was non-immune. The inoculation was made on December 16th. On the evening of December 27th the monkey was very quiet and on the morning of the 28th its temperature was subnormal and it was obviously moribund. It died in the afternoon. The African staff took a blood sample in the morning and another in the afternoon. They also took specimens of liver and other organs for histological study. Most unfortunately the writer was absent in Entebbe at that time, and no subinoculation of other animals was carried out, as the only European member of the staff who was at Hakitengya did not attend the autopsy. When the blood specimens were tested both the ante- and post-mortem specimens were found to be immune to yellow fever and, in addition, the pathology of the liver was very suggestive of an infection with the virus, in the late stage. It should be mentioned that in a yellow fever infection antibody appears early, sometimes within four days of the onset of the disease (Berry and Kitchen, 1931) and before virus has disappeared from the circulation, so there is nothing inconsistent in the monkey showing a positive protection test result from a specimen taken just before death.

It was concluded that the monkey died of yellow fever, following the inoculation of infected A. africanus. It seemed certain that, had subinoculations been carried out, as they should have been, the virus would have been isolated. As it was, it was felt that in a matter of such importance, an actual isolation of virus must be made for final proof, and that therefore the work must be carried on till this object was achieved. It was felt that now, however, the evidence against

A. africanus was so strong that a paper on the subject was warranted, and this was published by Haddow, Smithburn, Dick, Kitchen and Lumsden (1948). Thus, though the year ended with a major disappointment and the feeling that a chance had been missed, there was now a feeling of confidence in the sentinel programme, and a reasonable hope that with the next wet season the problem might be solved finally.

The other work of the year in Bwamba was the collection of monkeys, both in the mountains and in the lowlands. Included in the total was a series of mountain monkeys collected by Dick, to which reference has been made above, and also others collected by him in the lowlands. The lowland sample showed the same characteristics as before. Ten of the 26 specimens collected were immune. As all the immune specimens were adult or old monkeys, there was no indication of much activity by the virus among the local monkeys. All 18 specimens taken on the mountain were negative.

After the end of 1947 very few monkeys were collected in Bwamba though this work continued in other areas. It is therefore convenient to list the entire Bwamba sample at this stage, as follows:-

Age	Lowlands	Highlands
Unknown	1/5	-
Infant	5/16	0/3
Juvenile	0/22 (0%)	0/19
Subadult	7/51 (14%)	0/5
Adult	99/137 (72%)	0/8
Old	42/46 (91%)	0/5
Totals	154/277 (56%)	0/40

To sum up: during the year the reason for the failure of the sentinel programme to produce results was discovered. As soon as the remedy was applied, a sentinel underwent a yellow fever infection near Entebbe. At the end of the year a laboratory monkey in Bwamba died of yellow fever after an inoculation of A. africanus, but unfortunately an actual virus isolation was not made from it.

Work carried out during 1948

For some years it had been agreed between the Rockefeller Foundation, the Colonial Office, and the East African Governments that the Institute would eventually be taken over and run by the East Africa High Commission, and during 1947 it was decided that this change-over should be made at the end of 1949. The writer was at that time a member of the Foundation staff, and it seemed likely that he also would leave East Africa late in 1949, for a new assignment. In Bwamba the main uncompleted work was the "Bwamba Tree Survey", but there was a considerable unfinished programme in progress in other parts of Uganda. It was decided, therefore, that an effort should be made to complete the Bwamba work by the half-year, after which the station would be handed over to Dr. W. H. R. Lumsden, while the writer would work elsewhere. This left only one more rainy season in which to incriminate the vector. Owing to an injury and to an accumulation of work at Entebbe, it was not possible to begin field work in Bwamba till the middle of March. Work on the platforms had, however, been carried on by the African staff, and by the end of February the last monkeys had been taken from their cages and confined by the new method on the open platforms.

Lumsden was at that time carrying out a series of 100 catches in the sunset period (Lumsden, 1952), utilising five Mongiro platforms, and the writer took this work over temporarily and carried out 20 of them, Lumsden having gone to Entebbe. The rest of the time was spent on the 24-hour catches of the Tree Survey, which was being pressed forward as fast as possible, and in the supervision of the sentinel programme. There were now 39 sentinels in the canopy, one unsatisfactory platform having been abandoned. A certain amount of work was also still in progress in the Ntotoro Valley.

Early in the year Mrs. van Someren paid a visit to Bwamba to collect material intensively for systematic work, in order to round off the six years of collaboration on Bwamba material. At the end of the year this work was written up (Haddow, van Someren, Lumsden, Harper and Gillett, 1951). Even at that time the published information on the habits of

African Culicines was scanty in the extreme and as obviously there would be considerable delay before the analysis of the Bwamba Tree Survey and other surveys was complete, it seemed that a useful purpose would be served if what was known of the adult habits, larval habitat, etc., of the Bwamba mosquitoes was to be written up briefly, more particularly emphasising the biting habits. Mrs. van Someren added her extensive notes on the variations in colour pattern - inadequately dealt with in the existing textbooks. Other present and past workers in Bwamba contributed some records, Lumsden's records from huts, for example, being a valuable addition. The paper concerned is entirely for reference, containing in very condensed summary form what was then known of the 160 Bwamba forms. It does, however, contain what is probably the largest body of information on the habitat and biting habits of Ethiopian Culicines available up till the present.

Between the 2nd and 5th June the sentinels were bled as usual for the monthly protection test, and the bloods were sent to Entebbe. On 15th June a telegram was received, stating that the sentinel from Bundikitibo South had become immune. The station at Bundikitibo South is in a rather inaccessible part of the forest edge in the "Hakitengya Triangle". This was a serious disappointment as, in the case of the Entebbe sentinel which became immune in the previous year, there had been no overt illness, and it seemed possible that once again it might be too late to find infected mosquitoes. What had been hoped for was a serious or fatal infection, which would rapidly indicate local activity by the virus, without the delay of waiting for protection test results, etc. The monkey was at once brought in and bled for a retest (which eventually confirmed the first result). There being no spare monkeys in Bwamba at the time, a sentinel was brought in from another area, bled to show that it was still non-immune when brought into use, and sent out to Bundikitibo South on 16th June. The mosquito teams were working at the other end of the forest in the Mongiro area, and it was not possible to bring them in at once. Transport difficulties were considerable, as the only available truck was out of action, floods had done much damage to roads and bridges, and there was in any case hardly any petrol, a severe local shortage having followed a train derailment in Kenya. By 18th June, however, it was possible to begin intensive catching at Bundikitibo - mass catches at ground level by day, and evening catches on the platform. Two other sentinels were now brought in, bled for test and held in the laboratory to take inoculations of the Bundikitibo mosquitoes, as it was considered undesirable to inoculate these into the monkeys that had been receiving mosquitoes from Bwamba Tree Survey catches in the Mongiro area and elsewhere.

On 20th June the three current series of 24-hour catches in the tree survey were completed and, after a day's rest, the teams were moved to the Bundikitibo area. As some of the sentinel platforms in this sector had not yet been covered by the survey, the teams began series of 24-hour catches on the platforms, with controls at ground level, the stations covered being Bundikitibo South and the adjacent platforms at Bundikitibo North and Manjuguja Central. For a map showing the positions of the various platforms referred to here and below, reference should be made to the paper on this work (Smithburn, Haddow and Lumsden, 1949).

By this time, of course, all the monkey watchers had been warned to be alert for signs of illness in the sentinels, and extra supervisors were being used. All the mosquito catchers were ready to move as required to any point which might be indicated. Meanwhile, satisfactory numbers of mosquitoes were coming in from catches in the Bundikitibo area for inoculation. As the writer was alone in Bwamba at the time, the work was particularly heavy, and it was not felt that the catches could be extended to cover the other forest-edge stations of the Hakitengya triangle, unless there should be a definite indication of further virus activity.

On 25th June the sentinel from Manjuguja North was brought to Hakitengya in a moribund state. It was bled and then sacrificed. The gross pathological picture was typical of yellow fever with "boxwood" liver, gastric haemorrhage, delayed clotting time and serum with pronounced icteric tinge. Three sentinels were brought in and bled for test, and two of these, together with two groups of mice, were inoculated with serum from the dead animal, the third being sent out to replace it. It may be mentioned that yellow fever virus was subsequently isolated at Entebbe from some of these subinoculated animals. A telegram was sent to Entebbe asking for a truck to pick up the inoculated animals and pathological specimens, and evening catches were begun the same day on the platforms at Manjuguja North and Amagburugburu, a closely adjacent station. There now seemed a very good hope of obtaining an isolation from mosquitoes, as work had been begun actually on the day on which the sentinel died. The area to the south was, of course, already covered, and catching was now in progress at five stations - Bundikitibo South, Bundikitibo North, Manjuguja Central, Manjuguja North and Amagburugburu.

On the following day, 26th June, the sentinel from Tokwe River, near the point where the forest line of the Hakitengya Triangle joins the main road, was brought in sick, and with high fever. It was bled, but subinoculations were held over till the following day. On the 27th it was obviously moribund, and was sacrificed, when the gross pathological picture was found to be typical of yellow fever. Various sentinels were now called in from outlying areas, bled and held in reserve, as obviously a definite outbreak was in progress in the Hakitengya triangle. One of these monkeys was subinoculated with the serum of the dead Tokwe sentinel. It may be mentioned that yellow fever virus was subsequently obtained from the blood of this animal also.

On the same evening the monkey receiving inoculations of A. africanus from the Bundikitibo area became sick. Eventually it recovered, and it was shown that the sickness was not yellow fever. At the time, however, it was necessary to set it aside, and to bring in yet another for the inoculations. Spare monkeys and mice arrived from Entebbe later in the evening and, as further subinoculations now became possible, these were made during the night. Finally all the material was packed in the closed truck which had just arrived, was covered with mosquito netting, and sprayed with insecticide. The truck left for Entebbe at sunrise on the 28th and, as the material was presumably exceedingly dangerous, it was arranged that a police escort should be picked up at district headquarters in Fort Portal to accompany the truck to Entebbe, with a wireless check through stations on the road.

On the following day, the 29th, the three series of 24-hour catches in the infected part of the forest line were concluded. It so happened that similar series had not yet been carried out at the infected Tokwe River station or at that next to it, Bundimuguma, and arrangements were made to begin 24-hour catching at these two stations as soon as the teams had had a day's rest. In the evening Lumsden arrived from Entebbe to take part in the work, and a new programme was worked out, covering all the forest-edge stations of the Hakitengya triangle. Lumsden had been making experimental series of catches using fan-traps, and was anxious to try these out in the present work. He therefore took over the station next to the main road, Bubukwanga, and began stratified catches at a number of levels, with one trap on a pole above the canopy. The writer arranged to cover all the other forest line stations. Work at Manjuguja Central, some little distance from the main forest, was now discontinued. Series of 24-hour catches with ground-level controls were begun at Tokwe River and Bundimuguma on the 30th, while at the remaining stations, Mbango, Amagburugburu, Manjuguja North, Bundikitibo North, Bundikitibo South and Buhanda, catches were made on the platforms every afternoon and again every evening. Lumsden took over one monkey and all the A. africanus brought in were given an opportunity of feeding on this animal in the hope of demonstrating transmission by the bite of wild-caught naturally infected specimens. Apart from the 24-hour catches with their ground level controls and the stratified trap catches, work had to be confined to the forest canopy as there were not enough catchers to allow work at ground level as well. A point of considerable interest was that Lumsden's traps were taking quite large numbers of Phlebotomus spp. (none were taken in the baited catches) and a monkey was allocated to receive these.

On the 2nd July a telegram from Entebbe confirmed that the infections in the sentinels had been caused by yellow fever virus. Nothing of note occurred in the next few days. The Bwamba Tree Survey ended on 6th July and arrangements were made to wind up work on 9th July and to leave Bwamba on the 10th, after handing over the station to Lumsden.

On the morning of the 9th the monkey which had been receiving inoculations of A. africanus was found to be moribund and on sacrifice presented a typical picture of yellow fever. Mouse groups and monkeys were inoculated with serum and with liver suspensions, and arrangements were made to leave Bwamba the same night, in order to get these animals and the pathological material to Entebbe with the least possible delay. It may be mentioned that yellow fever virus was isolated from these sub-inoculated animals and thus there was final proof that A. africanus was a vector in nature - this proof being obtained on the last day of the six-and-a-half years during which the writer was in charge of the Bwamba Field Station. There now seemed a clear possibility that the monkey which had been exposed to the bites of A. africanus from the infected lot (it had been bitten by 91) might also have been infected (infection by bite usually shows itself later than infection by direct inoculation) and it therefore seemed best to take this animal to Entebbe also. It may be mentioned that after arrival there this monkey developed yellow fever (virus was isolated) but recovered, this proving that naturally infected A. africanus can transmit by bite. The monkey which had been receiving Lumsden's Phlebotomus collections was also taken to Entebbe, where it died of yellow fever after an incubation of hitherto unexampled (but not impossible) length. This was a finding of much interest, being the first time that yellow fever virus was recovered from an insect other than a mosquito. The significance of the finding cannot yet be appraised, as it is not known whether or not Phlebotomus spp. can act as vectors, or whether the isolation was merely made from an insect which had fed on an infected animal and whose stomach contained the infected meal. Laboratory work to determine whether Phlebotomus spp. can develop gland infections and transmit by bite is required.

Lumsden and Mr. W. A. H. Whittaker carried on the work until, in October, it was decided that it should be discontinued. Two further infections occurred in monkeys inoculated with suspensions of A. africanus and several infections occurred among the sentinels, one being at the previously unaffected station of Buhanda, at the extreme south-western corner of the Hakitengya triangle. The infections in sentinels may be summarised as follows:

1. Tokwe River
The first monkey died on June 27th. The replacement monkey occupied the platform till August 8th, when it had to be removed on account of intercurrent illness. The third monkey underwent a non-fatal infection between September 10th and 17th.

2. Manjuguja North
The first monkey died on June 25th. The replacement did not contract yellow fever, though it remained on the platform till September 7th, when it was removed on account of intercurrent illness. The third monkey was on the platform from September 12th till October 28th. Though it showed no overt sign of illness during that period, it was subsequently shown to have become immune, and thus must have undergone a subclinical infection.

3. Bundikitibo South
The sentinel at this station underwent a subclinical infection during May, or (at latest) the first days of June. The replacement monkey was sent out on 16th June, and underwent a subclinical attack between then and July 9th, by which time it had become immune. On July 22nd a third monkey was sent to this platform. It died of yellow fever on July 28th. A fourth monkey was put on the platform on the same day. It remained non-immune till the end of October, when the programme was finally closed down.

4. Buhanda
The sentinel died of yellow fever on July 27th. The replacement monkey remained non-immune till the end of October.

The isolations from insects were as follows:

1. From A. africanus

The first isolation was made from the 477 A. africanus caught between June 27th and July 8th. This isolation was made from the monkey which had been inoculated with suspensions of these insects, in 11 successive lots. The second isolation was made from a monkey which had been bitten by 91 of these mosquitoes.

The third infection occurred in a monkey which had received 26 inoculations, totalling 806 A. africanus, between July 12th and August 23rd. The infection was subclinical, there being no fever, and so was unsuspected. Accordingly, no attempt at isolation was made. The monkey was simply found, on routine test, to have become immune.

The next isolation was made from a monkey which received inoculations daily from August 24th to September 5th, the number of A. africanus being 254. This monkey died of yellow fever on September 6th, and virus was isolated from it.

The last isolation was made from a monkey which received 302 A. africanus in 14 lots between September 8th and October 3rd, when catches were discontinued. On October 6th it became febrile and, though it eventually recovered, yellow fever virus was isolated from a specimen taken on the first day of fever.

In addition to these monkeys two others which were used for inoculations of A. africanus, had to be withdrawn owing to intercurrent illness. Neither of these became infected with yellow fever virus.

2. From *Phlebotomus* spp.

   The monkey which became infected had been inoculated with a total of 133 *Phlebotomus* spp. As mentioned above, the incubation period was of remarkable length. Subsequent catches totalled 437 *Phlebotomus* spp., but virus was not again isolated from these insects.

The total number of *A. africanus* involved was not great, only 2,040, and the indication was thus that there was a great deal of virus activity and a high rate of mosquito infection in the area concerned. This view was supported by the fact that there were eight infections among the comparatively small number of sentinels at risk along the infected forest edge section of Hakitengya triangle. Thus there seems to have been a local outbreak or epidemic, much more severe than would have been suspected from a study of the age-incidence of immunity among the wild monkeys of the area. In spite of the obviously intense activity of the virus in the infected section, strains were recovered only from *A. africanus* and from *Phlebotomus* spp., though 5,258 other mosquitoes and bloodsucking Diptera, collected at the same time and from the same platforms, were also inoculated into animals.

The outbreak was of longer duration than had been expected. The experience of Bugher in South America was that virus rarely remains active in a given area for more than two months (personal communication). In the present instance, however, the first evidence of activity, the immunisation of the Bundikitibo sentinel, showed that virus had been present in the area in May or at the very latest in the first days of June, May being by far the more likely date. The last evidence was the October isolation from the monkey receiving *A. africanus*. Taking account of the usual incubation period, and making a liberal extra time allowance, it may be deduced that the earliest inoculation likely to have produced the infection was that of 21st September, 15 days before the fever. As work was closed down in October, the sentinels being returned to Entebbe, the further course of the outbreak is unknown. During its course, the virus was active along the entire forest-edge area of the Hakitengya triangle, the isolation from *Phlebotomus* spp. having been made from catches made at Bubukwanga, while the infection in the Buhanda sentinel occurred at the other end of the line. There was no possibility of telling from which station infected A. africanus had come as each lot was made up of a pool from the group of stations from which collections were being taken at the time concerned. Virus activity may well have extended westward beyond Buhanda but there were no sentinels beyond that point and no catches were made. On the other hand, no infections occurred in the main original sentinel line where the border of the main forest follows the road and, in this direction at least, the Bubukwanga area probably marks the limit of spread. All the infections in sentinels occurred in monkeys posted on the edge of the main forest, those stationed in other parts of the Hakitengya triangle all remaining uninfected.

A matter of considerable interest was that whereas in the case of practically all the previously isolated strains of yellow fever virus, infection in the rhesus monkey is almost invariably fatal, a considerable proportion of the rhesus used in the field and at Entebbe during the work under discussion survived the infection. Several of them did not even have a febrile reaction, though the fact that they became immune shows that they must have undergone an active infection.

One of the points advanced in favour of the view that yellow fever is an African virus which has been transported to South America is the fact that in African monkeys inoculation of virus usually gives rise to a very mild, self-limited infection, whereas in Asiatic and South American monkeys it is usually fatal. It is, of course, a general principle in the case of virus, bacterial or parasitic infections that the tolerant host is the natural host, and that the area where infections are mild is usually the area where the population has become tolerant or partially resistant by selection over a very long period. An extension of this point of view followed the present studies as here the virus was so mild that, although the rhesus is the most susceptible monkey known, many survived (though it may be noted that in various cases rhesus-to-rhesus passage immediately enhanced the virulence of the virus, as did passage in mice). It was now difficult to escape the conclusion that here yellow fever virus was being encountered in its mildest form because it was being obtained from one natural host, *Aëdes africanus*, taken in the natural habitat of the virus, the canopy of the African rain-forest, after repeated passage in other natural hosts, the African monkeys. For further information on this outbreak the published work (Smithburn, Haddow and Lumsden, 1949b) should be consulted.

This was the end of the main virus isolation programme in Bwamba, though the Field Station was maintained for another two years, some interesting work being done there, notably the observations on the sleeping-habits of monkeys by Lumsden (1951b) and Buxton (1951). The final picture in so far as yellow fever in Bwamba was concerned, was thus of a mild endemic disease of monkeys, transmitted from monkey to monkey by night in the forest canopy by a mosquito vector, *Aëdes africanus*. This mosquito, a tree-hole-breeding forest species, only occasionally bites man in his normal environment, as its main biting activity is arboreal and crepuscular. Various monkey species are, however, regular and determined plantation raiders, the redtail monkey (*Cercopithecus ascanius schmidti*) being particularly notorious in this respect. From infected monkeys the virus is passed on during the course of raids on plantations to a plantation-dwelling mosquito, *Aëdes simpsoni*, which breeds in plant axils, particularly those of colocasias, gonja bananas, and pineapples. These mosquitoes bite at all levels in plantations, even among the leaves of the largest plants, where the ripe bananas - which attract raiding monkeys - are to be found. It should be mentioned that even in a fatal laboratory infection in the rhesus the animal goes on feeding till shortly before death. In the case of African monkeys, feeding continues throughout the mild infection. That is to say, a monkey infected in nature would certainly remain with its usual band and would feed with them. From *A. simpsoni* the virus is passed on to man, who is bitten freely by this species in his plantations, though not in his huts. *A. simpsoni* is, incidentally, almost exclusively diurnal. The human infection is naturally most prevalent in the close vicinity of the main lowland forest, where the monkey population is highest. Both the human and the monkey infections are virtually - or completely - absent from the levels above about

5,000 feet in the Ruwenzori foothills, as at this level both vector mosquitoes disappear, being replaced by others in the appropriate ecological niches (*Culex musarum* is the dominant axil-breeder, and the dominant mosquito of the forest canopy after sunset is *Aëdes ruwenzori*). There is no evidence that the classical vector, *Aëdes aegypti*, plays any part in the transmission cycle in the area under consideration.

It is to be noted that though the epidemiological picture just outlined seems an adequate explanation of the known facts in and around the Semliki Forest, and while it is felt that probably a similar state of affairs is to be found in the main Ituri Forest of the Congo basin, it had already been clear for some time that it could not explain the situation in some other areas where work had been going on concurrently with the Bwamba studies. The situation in these areas will be discussed below.

WORK OUTSIDE BWAMBA

This is conveniently divided into three sections. First an account will be given of the entomological work and virus isolations. In this section present views on mosquito behaviour and problems of disease transmission will be discussed. As for some years past the study of yellow fever virus has been only one of numerous subjects under study at the Institute, it is not possible here to confine the discussion to this single virus as the work has rather general applications. In the second section the yellow fever survey work and epidemiological study in the wetter and more heavily forested Uganda localities will be discussed. In the last section an account will be given of the work on yellow fever in the drier areas of Uganda and elsewhere, and in certain of the forested localities in Kenya.

Much of the work now to be discussed is still in progress, and only some sections of it have been published. It is, therefore, necessary at several points to quote from annual reports of this Institute rather than from the regular scientific periodicals.

ENTOMOLOGICAL WORK AND VIRUS ISOLATIONS OUTSIDE BWAMBA

Much of the entomological work carried on outside Bwamba has consisted of further studies on the cyclical biting activity of mosquitoes and, more recently, on other cyclical aspects of mosquito behaviour. At present the main effort is concerned in the attempt to elucidate the mechanisms and stimuli underlying cyclical behaviour and to assess the relationship of such behaviour to the transmission not only of yellow fever, but of mosquito-borne infections in general. Much of this work has been carried out in the immediate neighbourhood of Entebbe, in the forests of the "Lake Victoria Belt".

A basic point in this work was that, while the biting behaviour of many species had been studied in detail in Bwamba, comparable studies from other areas were, at the time when the work was begun, non-existent. At a later date Mattingly's work on the biting cycles of mosquitoes in Nigeria (1949a and b) showed that in many species the behaviour patterns first noted in Bwamba held good in that area also. At the time, however, it seemed quite possible that biting cycles might differ from place to place and, in the case of the important species, it was essential that this should be determined. Another aspect of the work was the necessity for the study of species which are scarce or absent in the western forests.

The area where Entebbe is situated, just north of Lake Victoria, is a fairly flat and rather densely populated stretch of country, much intersected by arms of the Lake - sometimes very large - and by numerous swampy rivers and papyrus swamps. Much of the shore of the lake is occupied by reeds and papyrus, and behind this lies the narrow strip of forest so characteristic of the area. This in turn is backed by low, rather bare hills and by grassland. The hinterland presents a complicated pattern with numerous small forest patches, swamp areas, grassland and a great deal of African cultivation, all intimately intermingled. The bird and insect fauna of this area is considerable and very varied. Most of the larger mammals have been completely exterminated, but the small mammal fauna is extensive. Monkeys are poorly represented, only three species (Cercocebus albigena johnstoni, Cercopithecus aethiops centralis and Cercopithecus ascanius schmidti) being found, though admittedly their actual numbers are quite considerable, every small forest patch having its resident band.

Though the mosquito fauna of this area is less varied than that found in Bwamba, it is none the less extensive, and the numbers of mosquitoes taken in routine catches are consistently high. The rainfall is very well distributed, and there are, in fact, few occasions when breeding is severely affected by dry weather for more than a few weeks at a time.

As mentioned above, seven stations for sentinel monkeys were built during the period 1946-47 in the fringing forest of the lake, six at Zika and one at Kitinda, both near Entebbe. The forest in both areas is typical of the region. It consists largely of soft-wooded fast-growing species and is, as it were, in a state of unstable equilibrium, individual trees and small patches continually being cut by the local people and rapidly being replaced by new growth. Trees particularly characteristic of this type of forest are Trema guineense, Piptadeniastrum africanum, Pseudospondias microcarpa, Pycnanthus angolensis and Maesopsis eminii, with numerous Ficus and Albizzia spp., while in the swampy areas Phoenix reclinata, Raphia monbuttorum, Mitragyna stipulosa and Macaranga schweinfurthii are perhaps the commonest species of the edaphic climax. Everywhere the canopy is light, very irregular in height, and either discontinuous or barely continuous. In consequence, much light reaches the lower levels, and here the undergrowth is correspondingly dense, a tangle of vines and small trees overlying the ordinary flora of Marantaceous and Zingiberaceous plants.

The stations in the Entebbe area had a great advantage over those used in the Bwamba Tree Survey, in that they had in each case one platform in the canopy and one about half-way between ground level and the canopy, and thus it was possible to catch simultaneously at three levels at each station. The lower stations should not strictly be called "understorey" platforms as in forest of this type the understorey is far from well defined. It is convenient, none the less, to refer to them by this name. Five 24-hour catches were made at each of the seven stations, these 35 catches being referred to collectively as the "Entebbe Tree Survey". Not all the results of this series have as yet been worked out, and only some small sections have so far been published. It is not possible, therefore, to give a detailed account of the work here. Instead, certain points of significance will be picked out for attention, and the biting-habits of the important species, A. africanus, will be discussed.

It may be stated at the outset that in the great majority of cases the biting cycles observed near Entebbe closely resembled those already described for Bwamba, but in one or two instances there was a striking difference. Thus Anopheles implexus, which in Bwamba is to a very large extent nocturnal, was found to bite mainly by day in the Entebbe area (Haddow, 1954). This difference is not as yet understood. Further, in the case of the genus Eretmapodites, though in both areas the biting cycle was diurnal, differences of major degree were noted. In Bwamba the various species might begin biting at almost any hour of the day, but no matter at what time this activity began, it usually reached its peak in the first hour. At Entebbe, on the other hand, all species showed a marked tendency to concentrate their most active biting in the two hours before sunset. Once more this occurred regardless of the time at which biting began. These differences have been discussed more fully elsewhere (Haddow, 1956a).

The second main finding in the Entebbe work concerned the vertical distribution of certain species at different times of the day and night. A good deal of routine catching at ground level had been carried out previously in the area, and it had been noted that various species which in Bwamba are to a large extent confined to the canopy or to the understorey could be found in fair numbers by day in the undergrowth. The reason for this was at first puzzling, till it was realised that the light, irregular and often broken canopy of the lake-shore forests must admit a great deal more sunshine and wind than does the dense canopy of true lowland rain-forest. In addition, "sea-breezes" from Lake Victoria are of regular, almost daily, occurrence. It was concluded that perhaps the arboreal species might be driven down by these unfavourable conditions to take refuge in the shrub layer which, as noted above, tends to be very dense indeed. This was confirmed by a study of the vertical distribution of <u>A. ingrami</u> as shown by the 24-hour catches. The results (Haddow, 1954) showed that in the early morning the majority of specimens were to be taken in the canopy. Slightly later they had moved down to the understorey and by mid-morning the majority were to be found at ground level. In the afternoon an upward movement began, and by the period before sunset the main concentration was in the understorey. During the night numbers were small, but the canopy was the preferred level.

It seemed obvious that where a virus may be transferred from animals or birds in the canopy to man such vertical movements might be important, and consequently the figures for <u>A. africanus</u> were worked out in detail, but have not yet been published. They are now reported for the first time, and it is convenient to discuss here with them the various other findings for <u>A. africanus</u>.

Inspection of the records showed at once that when the levels were considered separately the biting cycle differed at each. In the canopy, where 1,115 specimens were taken 929, or 83 per cent of the total, were taken by night. The hour after sunset showed the usual sharp peak of activity previously noted in Bwamba, and 326 specimens (29 per cent of the total yield) were taken at this time. These figures are very similar to the results obtained in the Bwamba Tree Survey, which may be quoted for comparison, as follows: in the Bwamba work 2,178 specimens were taken in the canopy, and of these 1,774 (81 per cent) were taken by night, the single hour after sunset yielding 688 (32 per cent of the total for this level).

When the figures for the understorey were examined it was found that they presented the same general picture. The proportion of the total taken by night was, however, less. Thus of 562 specimens 370, or 66 per cent, occurred by night. The hour after sunset yielded 133 mosquitoes, 24 per cent of the total catch. An important difference here, however, was that a substantial amount of activity occurred before sunset. Thus in the two hours before sunset 84 mosquitoes, or 15 per cent of the total, were taken (in this period the catch in the canopy was equivalent to less than 5 per cent of the total yield). Further, when the records for the various platforms were examined individually, it was found that at two stations the highest level of biting activity in the understorey was reached in the hour before, not after, sunset. Unfortunately there are no Bwamba figures for this level to permit comparison between the two areas.

The picture at ground level was entirely different. Here there were no very pronounced peaks of activity but, broadly speaking, the main activity was diurnal instead of nocturnal. Altogether 194 specimens were taken in the 35 catches, and of these 148, or 76 per cent, were taken by day. There was no specially marked activity in the first hour after sunset, 16 mosquitoes, or 8 per cent of the total, being the yield at this time. The figures for the Bwamba Tree Survey are again closely comparable. Thus of 195 specimens taken at ground level 124, or 64 per cent, were taken by day. Once again the first hour after sunset was of little

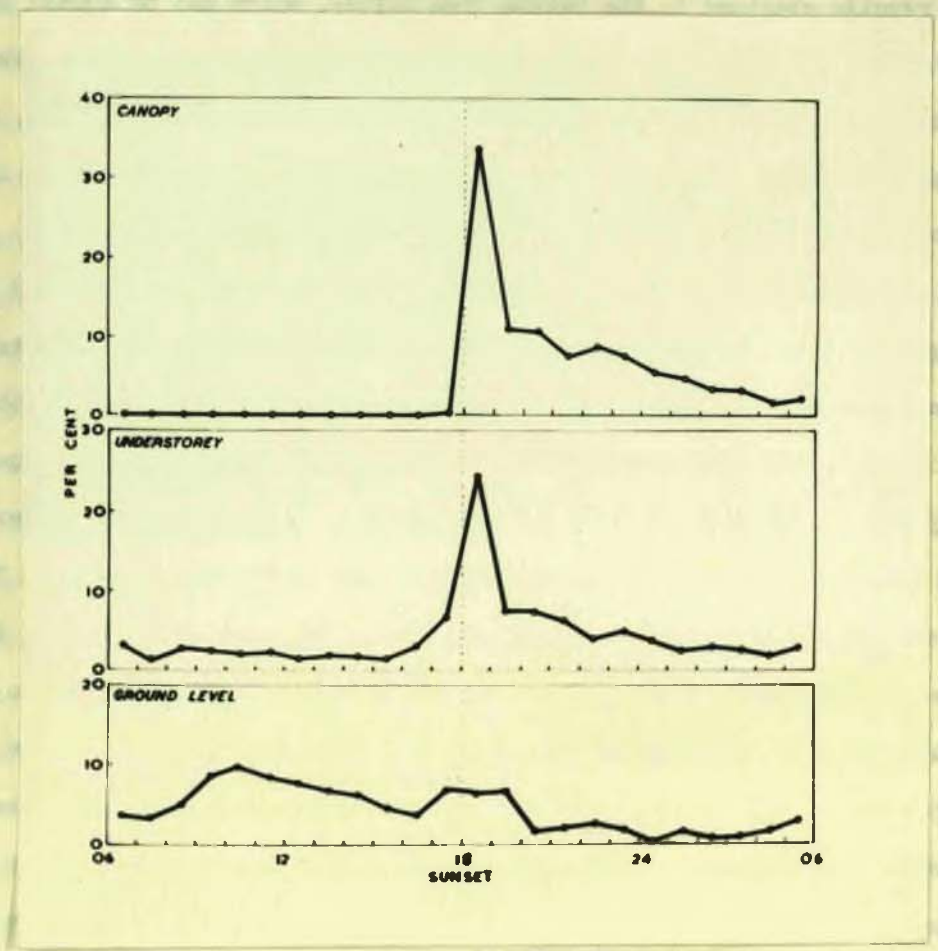


Fig. 6

The biting cycle of *Aëdes africanus* by level, as shown in the Entebbe Tree Survey (thirty-five 24-hour catches). The figures are geometric means, shown on a percentage basis to facilitate comparison.

importance, yielding 16 mosquitoes, or 8 per cent of the total. The biting cycles for the three levels at Entebbe are shown in Fig. 6. Here geometric means have been employed, and these have been shown on a percentage basis to facilitate comparison.

From these results it was concluded that, as in the case of A. ingrami, vertical movements might be taking place. Thus, of the small numbers taken biting at ground level the majority were obtained by day. In the period before sunset there was considerably heightened activity in the understorey, and after sunset in both understorey and canopy. To test this, the vertical distribution at different times of day and night was examined. The 24-hour period was subdivided into six periods of four hours each, these being related to the times of sunset and sunrise. As explained above, catch time was adjusted so that sunset always occurred at 1800 hours. The results were as follows:

Period	06/10	10/14	14/18	18/22	22/02	02/06
Canopy	67	35	84	620	226	83
Understorey	57	34	101	242	75	53
Ground	48	67	33	31	7	8

When the figures for each period are reduced to percentages of the total for that period to facilitate comparison, the results are as follows:

Period	06/10	10/14	14/18	18/22	22/02	02/06
Canopy	39	26	39	69	73	58
Understorey	33	25	46	27	24	37
Ground	28	49	15	4	3	6

These results are shown in Fig. 7. Here it is seen that in the period after sunrise (06-10 hours) the vertical distribution was indeterminate, the concentration of biting females being very similar at all levels. By the middle day period (10-14 hours) there seems to have been a movement downward, and there were actually more females biting at ground level than in the understorey or canopy. In the afternoon (14-18 hours) this movement had been reversed, and now the majority were to be taken in the understorey and canopy, the understorey being slightly, though probably not significantly, in the lead. In the period after sunset (18-22 hours)

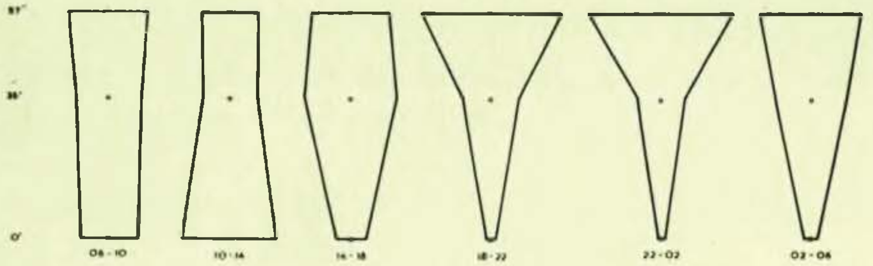


Fig. 7

The vertical distribution of Aëdes africanus at different times of the day and night, as shown in the thirty-five 24-hour catches of the Entebbe Tree Survey. The canopy figures are plotted at 57 feet, the mean height of the canopy platforms. Similarly, the understorey figures are plotted at the mean height of the understorey platforms, 35 feet. The distribution within each 4-hour period is on a percentage basis, to facilitate comparison.

the canopy was by far the most highly favoured level, and much the same applied during the middle night period (22-02 hours). In the period before sunrise (02-06 hours) this distribution was less well marked - possibly a move toward the indeterminate distribution found after sunrise. It is a striking observation that, of the 1,871 specimens taken in the whole survey (all levels combined) 620, or 33 per cent, were taken in the canopy in the four hours after sunset and 326, or over 17 per cent, in the single hour after sunset at this level. Thus, as in the case of A. ingrami, there seems to be definite evidence of vertical movements by A. africanus in the forest belt of Lake Victoria, and in this connection it is interesting to note that Mattingly (1949a), working near Lagos, had suspected that such movements might be occurring in that area also. The same is probably the case in the rain-forests of the west, as in Bwamba biting at ground level was also diurnal rather than nocturnal. Here, however, any such movements are likely to be much less marked as the closed and much heavier canopy probably gives far better day harbourage. There is some support for this view in the figures. Thus in the Entebbe Tree Survey (35 catches) 194 were taken at ground level. In the Bwamba Tree Survey the number (195) was almost identical, though here the number of catches (240) was almost seven times as great. Looking at the figures in a different way, it is found that at Entebbe the ratio of A. africanus caught in the canopy to those caught at ground level was about 6:1. In Bwamba it was about 11:1.

It is surprising, in view of these findings, that the incidence of immunity to yellow fever among the Africans of the Entebbe area should be so low. It is of course true that they are in no way to be described as forest people. When passing through small forest patches on their way to and from the lake they use well defined paths. On the other hand, they do cut timber for many purposes, and they clear bush and forest to make way for their wasteful shifting cultivation. Under such circumstances the men at least must often be bitten by A. africanus. The situation is puzzling, and calls for further study.

Another way in which the behaviour of A. africanus tends to be influenced by the irregular and sparse nature of the canopy at Entebbe is well shown by comparing the figures for two of the stations, Kitinda and Zika III. The Kitinda station is very close to the lake shore, and tends to be affected by wind at the peak period just after sunset. Wind is known to have an unfavourable influence on the biting activity of A. africanus (Haddow, Gillett and Highton, 1947; Haddow and Mahaffy, 1949). In the five catches here there were four in which wind affected seriously the yield from the canopy. The better protected understorey station was less affected, and in three of the catches concerned actually yielded a higher catch than the canopy, as is shown by the following figures for the numbers taken, by level:

Catch	1	2	3	4	5
Evening weather	Warm, Calm.	Cool, Windy.	Cool, Windy.	Cool, Windy.	Cool, Windy.
Canopy	51	29	9	9	3
Understorey	18	23	17	17	7
Ground level	1	3	2	0	2

The results from Zika III, which is a well sheltered station, and where weather was fair throughout the catch series, are in striking contrast:

Catch	1	2	3	4	5
Evening weather	Warm, Calm.	Warm, Calm.	Warm, Calm.	Warm, Calm.	Warm, Calm.
Canopy	119	51	12	20	49
Understorey	31	20	3	5	7
Ground level	4	15	0	3	3

Generally speaking, apart from cases such as that of Kitinda just described, A. africanus has shown perfectly regular and typical behaviour in the canopy at Entebbe. The exceedingly short nature of the biting peak at this level was clearly demonstrated by Lumsden (1952) who subdivided catches made at the sunset period into 10-minute intervals. The writer's experience has been that, if catching is proceeding at a single level only, the simplest way to subdivide the catch is by 1-hour periods or by 1-minute periods. The latter type of catch is conducted as follows: The supervisor has a book, one page per hour, already divided up into minutes. The tubes have labels, but no numbers. As a mosquito is caught the catcher

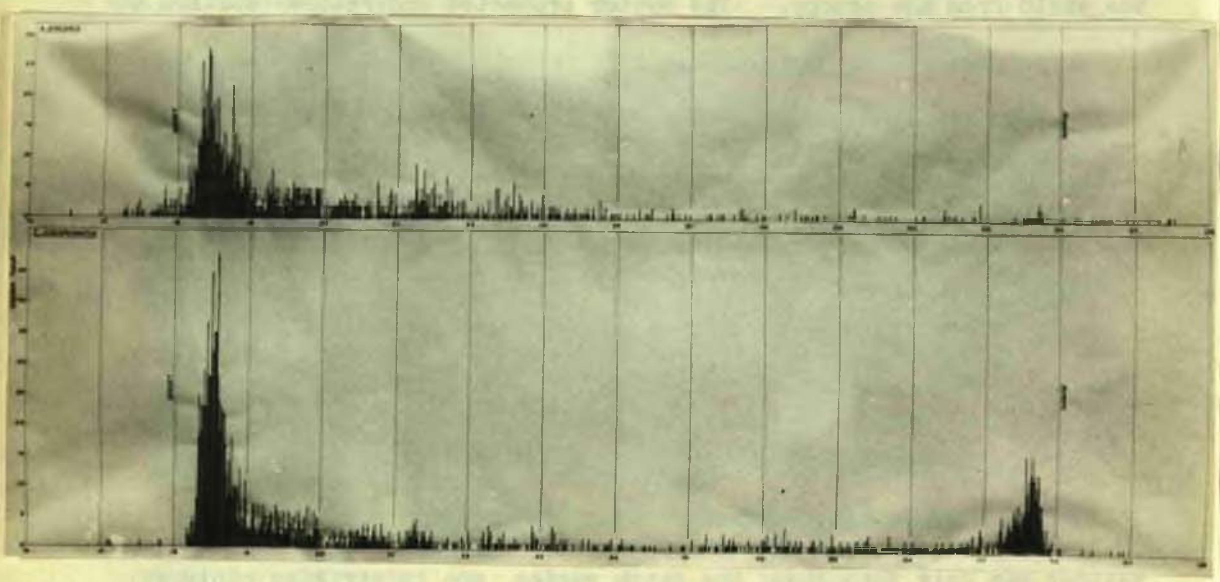


Fig. 8

The biting cycles of Aëdes africanus and Taeniorhynchus fuscopennatus, as shown by sixty 24-hour catches at Zika No. 4 platform, minute-by-minute results. To facilitate reproduction (the original graphs are very large) most of the daylight hours have been omitted. This results in the loss of only 1 T. fuscopennatus and 13 A. africanus.

calls out to the supervisor, who calls out its serial number which the catcher writes down on the tube. The supervisor simultaneously enters the number opposite the appropriate hour and minute in his book. The general conduct of 24-hour catches has been discussed elsewhere (Haddow, 1954) but the method now discussed was developed subsequently, during studies on Culex sitiens on the Kenya coast. It has the great advantage that the results can be regrouped by any desired time interval, and in many cases it gives an exceedingly clear picture of biting activity. Large numbers of specimens are, of course, required. The method has been used in the last years in making detailed studies in the forest canopy in the original station at Zika IV. Sixty 24-hour catches were carried out there by this method and they have given by far the clearest picture to date of the biting pattern of A. africanus. The results, together with those for another common species, are shown in Fig. 8 and will receive further comment below.

As knowledge concerning the cyclical biting-behaviour of mosquitoes and Tabanids has increased, an increasing amount of work has been devoted to attempts to explain the underlying mechanisms. In this connection it is necessary to discuss in some detail a theory advanced by Lumsden (1952) and supported by him subsequently (1955b), as the view he puts forward would, if substantiated, influence enormously the whole approach to the question of transmission, not merely of yellow fever, but also of other mosquito-borne diseases. Lumsden carried out a long series of detailed catches in the sunset period at Mongiro, Bwamba, the more critical hours being divided into very short intervals of ten minutes each. These catches were carried out on the platforms built for the Bwamba Tree Survey. Lumsden's main finding was that many species show a short peak of activity just after sunset. He differentiates biting-cycles into types like that of A. africanus, where this is the main peak of activity, and types like that of T. africanus and A. gambiae, where it is a minor peak, and where the main peak of activity occurs much later.

Lumsden suggests that nocturnal species are inhibited from biting by daylight, but that during the crepuscular period they are "released" as the light fades. So far, his view is almost certainly correct. He believes that those individuals which bite just after sunset represent the older age-grades - those which are ready to bite at once when conditions become favourable. These are, he believes, the mosquitoes which have already fed one or more times. Those which feed later at night are, according to his view, young females which have probably been mating in the sunset period and so are not ready to feed at that time. Lumsden states that the first blood meal differs from all others in that it is preceded by mating. Without wishing to dwell particularly on the subject of mating, it may be stated that this is not always true. Various species do not mate till the first blood meal has been taken, for example, Aëdes albopictus (personal observation) and Eretmapodites chrysogaster (Gillett, 1954).

Lumsden holds that in the case of the small-container breeders populations are probably never very large and that therefore females must survive several oviposition cycles in order to maintain the population level. Thus there will be a high percentage of old females, ready to feed just after sunset, and these will build up a major wave of activity at this time, as in A. africanus. The subsequent - much smaller - numbers biting later in the night, will be made up of young females coming to bite for the first time.

Where breeding places are extensive and large populations can be built up, as in A. gambiae, the number of ovarian cycles per mosquito will, he believes, be much smaller, and the percentage of old females correspondingly less. These old females will form the small peak of activity shown in the post-sunset period, while the main, gradually increasing wave which builds up to the principal peak later in the night, will be made up of large numbers of young females biting for the first time. Examples of these two main types of curve, as exemplified by A. africanus and A. gambiae have been shown elsewhere (Haddow, Gillett and Highton, 1947).

At first sight Lumsden's theory appears a most attractive one, neatly grouping the various forms of biting cycle on a rational basis. The theory implies that by night the actual transmission of mosquito-borne organisms (as opposed to the picking up of infection by younger insects) must occur mainly in the hour after sunset, when the older age groups, it is suggested, reach their peak activity. It is known, in fact, that in another group of bloodsucking insects (Simulium spp.) different age grades do tend to bite at different times. In the case of S. damnosum support seems to be forthcoming for Lumsden's theory, as here old females are commonest during the earlier part of the biting cycle (Lewis, 1956). On the other hand, the exact reverse is the case in S. ornatum, as here young females predominate during the earlier part of the cycle, and old females toward the end (Davies, 1955).

Various workers in the same field have been unable to accept Lumsden's theory in so far as mosquitoes are concerned and, in the present writer's view, it cannot be reconciled with the known facts. As the entire problem of transmission is here involved, it seems of importance to review the evidence.

Lumsden's catches were confined to the period around sunset, and this has led him to draw conclusions concerning the seasonal incidence of certain insects at Mongiro which conflict with the results of extensive series of 24-hour catches carried out in the same locality. That in the case of A. gambiae a fallacy has arisen from expanding the findings made in the sunset period to cover the whole night's catch has already been shown above. The same applies to deductions concerning the seasonal incidence of an insect of another group, Chrysops centurionis. Lumsden maintains that this species has only one main emergence period per year, during late March and early April (i.e., early in the main rains), yet exactly comparable series of catches carried out at the height of the rains and of the dry weather at Mongiro showed that in the wet season of 1944 C. centurionis was much less abundant (only one was taken) than it was during the exceptionally severe dry season of 1945, when 24 were taken (Haddow, Gillett, Mahaffy and Highton, 1950). The explanation is that Lumsden's catches were circumscribed not only in time but also in level, being confined to the canopy, whereas it has been shown in the paper just cited that in hot dry weather this Tabanid tends to move down from the canopy to the understorey. As in the case of seasonal incidence so also with biting activity, it is necessary, before drawing conclusions, to look at the whole picture - i.e., to make catches lasting throughout the 24 hours, and to make them at various levels.

Accepting the fading of daylight as very probably a release mechanism for crepuscular and nocturnal feeders which have been inhibited by full daylight, the reverse should apply to diurnal feeders which, equally probably, are released by its return. In the case of small-container breeders one would expect, if Lumsden's view were accepted, a major wave of activity just after sunrise. The writer does not, however, know of a single species where this occurs. A few examples may be quoted here of the behaviour of small-container breeders which bite by day and which show patterns differing strikingly from that just postulated. A. simpsoni is active throughout the day, showing biphasic activity in plantations, or a single main wave of activity in the afternoon in forest. A. apicoargenteus, as shown by recent extensive series of catches near Entebbe, shows steadily increasing activity from sunrise till midday, after which a gradual decline till sunset follows. Eretmapodites spp. show entirely different patterns of behaviour in Bwamba and in Entebbe (an inexplicable state of affairs if Lumsden's view be accepted) but in neither area do they show a large wave soon after sunrise (Haddow, 1956a). Even admitting that the period just after sunrise falls within the coldest part of the 24 hours, and that the low temperature might inhibit biting, the major wave should surely follow rather later - say by 09 or 10 hours - by which time the temperature has risen markedly. No case is known, however, of an East African mosquito showing its most pronounced activity at this time.

The above discussion is to some extent deductive, and it may be well to discuss a particular mosquito, Anopheles gambiae, in more concrete terms, and thereafter to consider some swamp breeding species which build up enormous populations seasonally and which, if Lumsden's view be accepted, might be expected to show a small peak of activity in the post-sunset period (old females) followed by a gradual build up to a major peak later in the night (young females).

The first point is that the small peak of activity shown by A. gambiae in the hour after sunset is very far from constant. This species may begin biting at any hour between sunset and sunrise. Combining the 40 catches of the Mongiro-Mamirimiri surveys of 1944-45 and the 240 catches of the Bwamba Tree Survey, it is found that A. gambiae was taken in the canopy on 117 nights. On only 41 of these nights (approximately one third) did biting begin in the hour after sunset. On another 41 nights no biting took place till 4 or more hours after sunset. This shows unequivocally that the post-sunset peak in the canopy upon which Lumsden bases his argument, is far from being the regular occurrence which that argument implies. This has been discussed in more detail elsewhere (Haddow, 1954). An equally significant finding was that at different levels the biting cycle varied considerably in detail, though always nocturnal and always with the main wave of activity occurring shortly before sunrise. In the first discussion of the Mongiro-Mamirimiri catches (Haddow, Gillett and Highton, loc. cit.) the catches from different levels were combined, as the differences seemed of a minor order. More detailed subsequent analysis, however, combined with the use of the geometric mean (Haddow, 1954) has shown that when the figures are considered on a percentage basis the highest (canopy) platform at Mongiro shows a very acute peak of activity before sunrise, and that with decreasing height above ground this becomes progressively less well marked. Further, the post-sunset peak upon which Lumsden bases his argument, while relatively well marked in the canopy and understorey results, is hardly apparent in the figures for the lowest platform (16 feet) and not at all in the figures for ground level, which is the normal habitat for this species, and the level where over 70 per cent of any stratified catch of A. gambiae will almost certainly be taken.

Terminating the paper under discussion Lumsden says "The experimentum crucis to test the validity of the hypothesis now advanced has not yet been performed. It is the estimation of the age of the mosquitoes coming to feed period by period through the night by means of ovarian examinations". It so happens that the writer had carried out such work at Kisumu, in 1940, on A. gambiae entering huts. The total catch of all

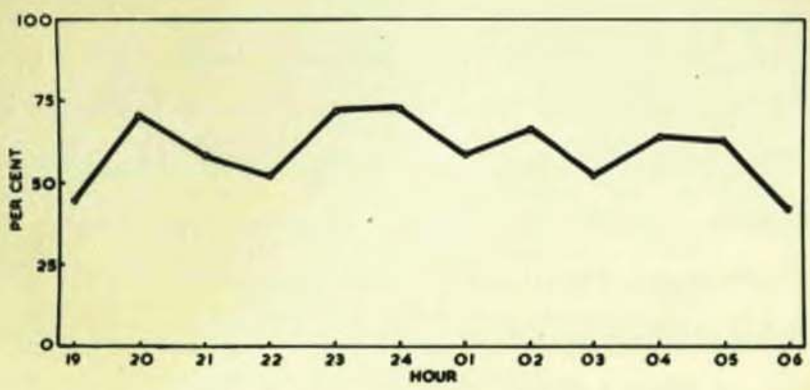


Fig. 9

The entry of young Anopheles gambiae, with ovaries in Stage I, to a hut at Kisumu, as shown by dissections of specimens taken in 20 all-night catches. The figure for each hour shows the Stage I females as a percentage of the total A. gambiae taken in that hour.

species during 20 all-night catches was dissected, but only a brief comment on the first 18 of these has been published (Haddow, 1942a). It is now, however, necessary to discuss the matter further. In Anopheles generally the first feed is taken when the ovary is in Stage I, as described by Christophers (1911). After this the ovary never returns to Stage I, and a female coming to feed after her first or subsequent oviposition will have an ovary early in Stage II. A female with a Stage I ovary is, therefore, recognisable unequivocally as a young mosquito feeding for the first time. The percentages given below and illustrated in Fig. 9 show quite definitely that throughout the night the young unfed females form a very constant proportion of the total catch in an African hut. Gillies (in press) suggests that the first hour after sunset should be omitted, as here the result is affected by mosquitoes which have been resting in the hut by day and have not yet left, and similarly that the last hour before sunrise should be omitted as it is influenced by mosquitoes entering for shelter, and not to feed. Even when these are included, however, it is seen that in every hour of the night the percentage of mosquitoes feeding for the first time is high, the results being:

Hour	Total A. gambiae	Total Stage I	Per cent Stage I
19	42	19	45%
20	17	12	71%
21	17	10	59%
22	21	11	52%
23	32	23	72%
24	29	21	72%
01	32	19	59%
02	49	33	67%
03	44	23	52%
04	39	25	64%
05	27	17	63%
06	69	29	42%
Totals	418	242	58%

It will not escape attention that the percentage of young females in the hour after sunset was virtually the same as that in the hour before sunrise. These figures have recently been strengthened by the work of Gillies (in press) who has shown that, if the night be divided into three approximately equal periods, his catch results show the following distribution of Stage I females:

Period (Hours)	Per cent Stage I A. gambiae
1830-2200	36%
2200-0200	28%
0200-0530	29%

It will be noted that the figures, while much the same from period to period, do show the highest percentage of young females in the early night when, according to Lumsden, old females should predominate.

Similar results have been obtained by Senior White (1953). His evening catches extend only to the second hour after sunset, but this is adequate for the present discussion. In the hour after sunset he took 2,237 Anopheles aquasalis, a species with extensive breeding grounds. Of these 413 (18 per cent) were in ovary Stage I. In the succeeding hour he took 1,190, of which 159 (13 per cent) were in this stage. Thus the percentage of young females was higher in the hour after sunset than in the succeeding hour. A similar result was obtained with A. maculipalpis, though here the sample was rather small. Thus the results of direct experiment here again disagree with Lumsden's hypothesis which, it must not be forgotten, depends entirely on deduction.

A second case is that of T. fuscopennatus, a swamp-breeding species which, along the northern shores of Lake Victoria, occurs in vast numbers at certain times of year. It is, in fact, the main "nuisance mosquito" of this area. The biting cycle as seen in the canopy has recently been described (Haddow, 1956b). Just after sunset there is a very striking peak of activity (Fig. 8) of the same type as that shown by A. africanus, but even more pronounced. Thereafter there is very little activity till the hour before sunrise, when a minor peak occurs. It may be added that the Entebbe Tree Survey showed that the cycle, as in A. gambiae, varied from level to level. Thus in the understorey the sunset and sunrise peaks were much less well marked, and more mosquitoes were taken in the inter-vening period. At ground level the sunset and dawn peaks were quite small, and fairly large numbers were taken during the rest of the night, not to mention quite substantial numbers by day. These differences are quantitative rather than qualitative, but it may be mentioned that a series of catches made in a banana plantation actually contiguous with the forest and another in a hut only eighty yards from its edge showed an entirely different picture. In neither of these did the sunset peaks appear. In the hut biting was most active after midnight. In the plantation the curve was merely nocturnal, with no particularly well marked waves of activity. T. fuscopennatus is a mosquito with a long flight-range, and its extensive breeding places could not in this instance be considered as closer to any one of the catching sites than to another (Lumsden, in the second of the papers mentioned above has suggested that differences in the percentage of young and old females at different distances from the breeding-sites may account for differences in biting-patterns).

As of all the mosquitoes of the Entebbe area T. fuscopennatus is the one which should, if Lumsden's view is correct, show a biting-pattern resembling that of A. gambiae, and as in actuality the pattern - in the canopy at least - closely resembles that of A. africanus, a small-container breeder, it was decided that more detailed investigation was desirable and that dissections should be made to determine the percentages of young females biting at different times of night. Almost at once a difficulty was encountered, as it was found that this species does not come to bite till the ovaries have reached early Stage II (presumably a nectar feed is taken before the blood meal) and thus it is impossible to pick out the young females feeding for the first time. The only method of approach seemed to be to reverse the process, and to try to find the percentage of old females. After a female has laid, one or two "relict eggs" may remain in the ovary. Naturally, when an immature ovary is found containing such eggs (they are exceedingly obvious) it may be stated definitely that the female is an "old" specimen - i.e., one initiating a second or subsequent ovarian cycle. To determine what percentage of the females of the species retained eggs in this manner, a large number were put up in individual tubes, with water for oviposition. After the egg-rafts had been laid the females concerned were dissected, to a total of 200. Of these 32, or

16 per cent, were found to contain relict eggs, and this was taken as a standard. The females taken in three 24-hour catches at Zika were now dissected. A total of 283 specimens was taken, all of which had ovaries in Stage II. The catch from the hour after sunset and that from the hour before sunrise were large enough for individual treatment. That from all the intervening hours was combined, as only thus could a number large enough for analysis be made up. The results were as follows:

Period (Hours)	18-19	19-05	05-06
Stage II	131	59	73
Stage II with relict eggs	9	5	6
Total	140	64	79
Per cent with relict eggs	6.4%	7.8%	7.6%

Thus in the three periods there was a very constant proportion of females with relict eggs. If the figure obtained in the previous discussion be accepted, then these will be considered to make up 16 per cent of the old females with at least one oviposition cycle completed. From this it may be deduced that the numbers of old females in the three groups represented about 40, 49 and 47 per cent of the total catch.

Gillett (in press) has approached the subject from a different standpoint. Swamp-breeding mosquitoes often become infested, at the time of emergence, by the parasitic larvae of Hydrarachnid mites. Many of these - probably almost all - return to the water at the time of the first oviposition. Gillett, working with T. africanus, has shown that most of the mosquitoes carrying mites are obviously freshly emerged specimens, with unworn scaling and undamaged wing-fringes. He has further shown that the percentage of females with mites forms an exceedingly constant proportion of the total catch hour by hour throughout the night. His conclusion is that young females form a large percentage of the biting population at all hours. This is in direct conflict with Lumsden's hypothesis.

Thus in all the cases known to the writer where work has actually been carried out to test its validity, Lumsden's theory has failed to explain the observed facts. On the other hand, while rejecting it, the writer feels that a suggestion of value may lie in the proposition that different population groups behave differently. This view, actually, was first advanced by Mattingly (1949a), some time before Lumsden's more detailed theory was advanced. In this broadly outlined form the idea seems worthy of further study, and at the moment there is not enough evidence to indicate what the result might be. In the case of A. longipalpis, however, some work has been done and here there seems to be some evidence that perhaps different sections of the population - whatever they may be - do show different biting patterns (Haddow, 1954).

With reference to the possible influence of microclimate on biting activity, the writer has long since abandoned the idea that a mosquito is actively conditioned to bite by suitable microclimatic conditions. In place of this the view is now held that if the microclimate is unfavourable biting will be inhibited. When conditions become favourable, the insect is "released". Whether or not it will then bite may, it is believed, depend on one of many factors - its own physiological state probably being one of the more important. On the other hand, in view of the evidence now available, it is hardly to be doubted that microclimate must have a profound influence on cyclical activity, light being almost certainly the most important single factor.

Some examples may be quoted as follows: Colles (1956) has shown that the size of night catches of Anopheles leucosphyrus is directly related to the amount of moonlight, and Ribbands (1946) has found a similar relationship in Anopheles funestus. Senior White, Lewis and Lee (1953) have pointed out that there is a critical light intensity above which swarming and mating in Anopheles aquasalis do not occur. Swarming begins 10-15 minutes after sunset, when the light incidence does not exceed five foot candles, and mating occurs when it has dropped to less than one foot candle. Nielson and Greve (1950) have produced a very detailed study of swarming, with particular reference to Aëdes cantans. They have shown that "the ascent" to form what they call "top swarms" (i.e., high swarms over trees, etc.) seems to be dependent on the afternoon drop of temperature to a particular level. The subsequent break up of the swarms, however, does not apparently depend on temperature but occurs when the light falls below a certain intensity. It is most interesting to note that in the morning the process is reversed. The swarms are formed when the light reaches a certain intensity and break up when the temperature passes a certain level. It is interesting to note that the threshold values differ in different species, so that there is a succession effect in the evening which is reversed in the morning. Two nectar meals were taken, the one before the evening swarm and the other after the morning swarm - it is known that sugar forms the main source of flight energy, the blood meal of the female being simply a source of protein to permit egg development. Feeding on flowers has not been studied much so far, but here also microclimatic factors may come into play, as Bro Larsen (1948) has shown that the meals are taken, in Denmark, at the periods corresponding to the lowest wind force and highest humidity. One of the most striking demonstrations of a microclimatic influence controlling mosquito behaviour is that given by Wellington (1945a) and deals with stratified catches of Culicines in Toronto. He has shown that the female of Culex sp. has a lower minimum flight temperature than has the male. When the temperature of the air column (up to about 60 feet) is fairly high, males are fairly evenly distributed. Females remain in the lower levels, presumably to feed. On certain nights "ground inversion" occurs, the ground level temperatures being lower than those above. In such cases the males are confined to the warmer, upper levels, but females may still be found below. On cold nights (under 60°F) with ground inversion, no males are to be found and females, though still active, are now confined to the upper, warmer levels. This is a particularly clear and satisfactory demonstration of the profound influence which may be brought to bear on mosquito activity by a single microclimatic factor.

With increasing knowledge of mosquito biting-behaviour, a problem which has begun to come into prominence is the observation that in different environments the same species may show entirely different cyclical behaviour. This has been found to be the case in almost every species so far adequately studied. An example is given above, in the case of T. fuscopennatus, where the forest cycle varies from level to level and

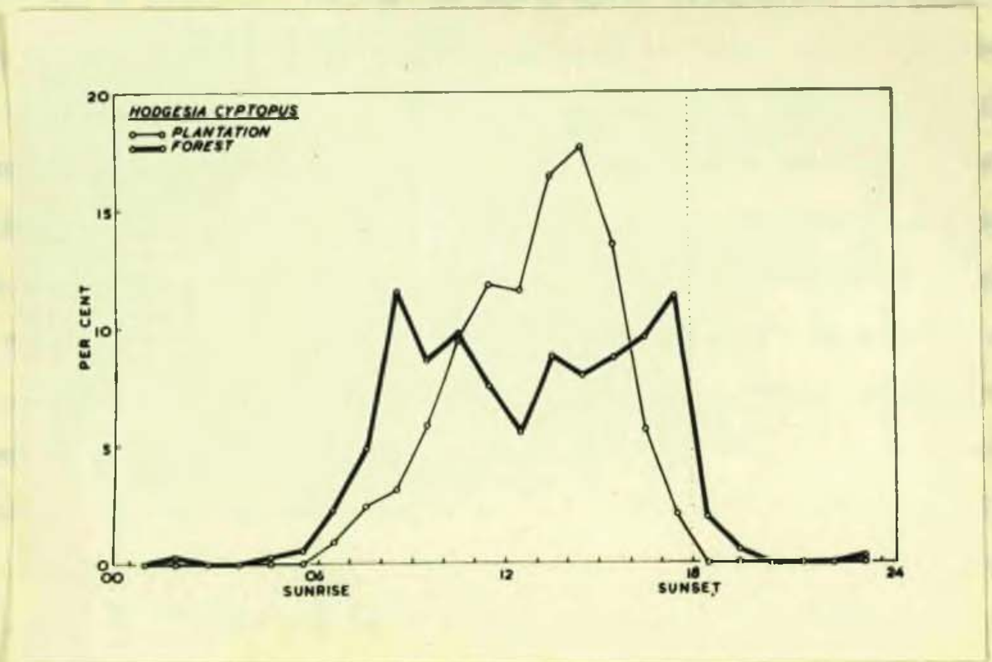


Fig. 10

The biting cycle of Hodgesia cyptopus in the forest at Zika near Entebbe, and in a banana plantation contiguous to the forest.

differs completely from that found in banana plantations and in houses. A fine series of examples is given by Deane, Causey and Deane (1948) who studied the biting cycles of Anophelines inside and outside houses. In every case in which adequate numbers were obtained there were striking differences. Particularly notable was the case of Anopheles aquasalis, an important species which in the open air bites mainly before midnight and in houses mainly after midnight. A parallel has recently been obtained on the Kenya coast, where work on Culex sitiens, carried out by the writer in collaboration with the Division of Insect-borne Diseases, Kenya Medical Headquarters, has shown a close resemblance between the biting-habits of this species and those of A. aquasalis. As a final example, the case of Hodgesia cyptopus may be mentioned. This tiny, diurnal mosquito was studied at Zika, both in forest and in a banana plantation which was actually contiguous with the forest. The cycle in the plantation has been discussed in detail elsewhere (Haddow, 1954). That in the forest has not yet been published. Detailed figures will not be given here, but it will be seen from the graph (Fig. 10) that in the plantation there was a single marked peak of activity in the mid part of the day, while in the forest the cycle was biphasic, with peaks in the morning and afternoon. This final case is mentioned as there is here a clue to the difference in behaviour. Within the forest edge incident light on the forest floor also shows a biphasic trend, closely corresponding to the cycle of A. cyptopus (at midday the almost vertical equatorial sunlight is partly obscured by the crowns of the trees, while in the morning and afternoon it slants in more freely). In the opener banana plantation, on the other hand, it rises steadily to a maximum in the middle part of the day and then declines.

These examples will serve to show that explanations of biting-patterns must allow for the interaction of many factors and that no single, simple explanation is likely to prove satisfactory. Certainly, in our present state of knowledge, it seems that microclimatic factors must often play a considerable part in determining behaviour. In this connection it is of interest to consider the work of Corbet and Tjønneland (1955) on the flight activity of East African Trichoptera. Among the twelve species they studied, several showed "eo-crepuscular" patterns with dawn and sunset peaks of activity, resembling that of T. fuscopennatus biting in forest. Others showed a single crepuscular peak like that of A. africanus. These results are of special interest, as most of the insects studied live for a very short time in the adult state - sometimes a single night only - and as many of them probably do not feed as adults no question of age-grades or of feeding cycles can enter. We are left with the impression that here external environmental conditions must play a large part in imposing these patterns. Work on yet other groups supports this view. For example, Johnson (1953) has pointed out that in the diurnal cyclical flight of Aphids calm is essential for the take-off, and perhaps for re-alighting. More recently still, Dunning (1956) has shown that the well-defined diurnal cycle of emergence in the beet fly, Pegomyia betae, is probably governed by microclimatic factors.

It is only recently that work on cyclical biting behaviour has progressed sufficiently to permit the study of other cyclical aspects of mosquito life to be begun at Entebbe. There is, none the less, a very strong conviction among most of the workers at this Institute that many problems of disease transmission will remain obscure till the various activities which are probably periodic have been studied and understood.

Work by Bro Larsen (loc. cit.) and by Nielsen and Greve (loc. cit.) had shown that flower feeding was periodic in mosquitoes, and there was a strong suggestion from the results of various workers that swarming and mating are also periodic and essentially crepuscular. These activities, however, are very difficult to observe in captivity, and many species will not mate in cages at all. The first activity selected for study has therefore been oviposition, and this work, though begun only three months before the time of writing, has already been very fruitful. As it opens a new field the use of subordinate staff has been avoided, and all the work has been carried out by the writer and J. D. Gillett in collaboration. As observation goes on by day and by night, usually in continuous spells of five days and five nights, it has not been possible

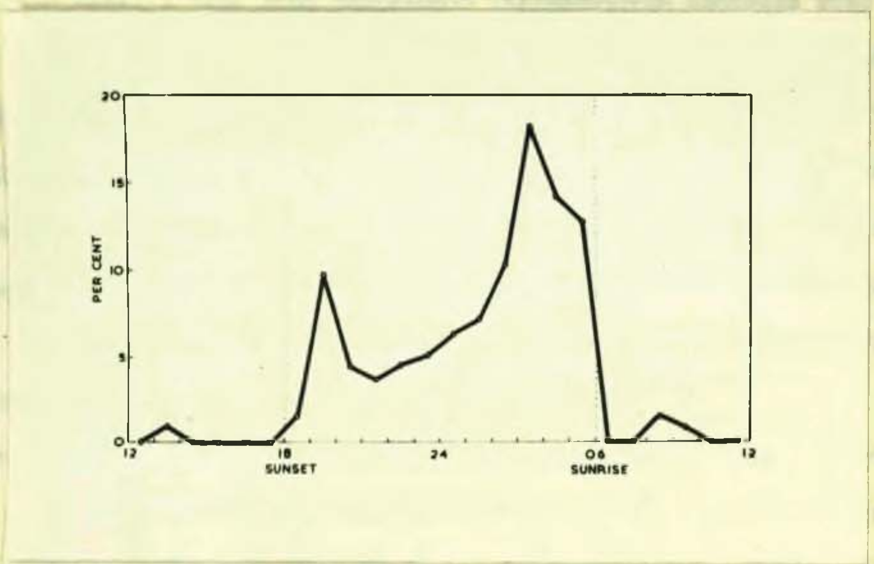


Fig. 11

The oviposition cycle of Taeniorhynchus fuscopennatus. The figures show the geometric mean number of rafts laid per hour, and are shown on a percentage basis to facilitate comparison with other species.

to carry out all of it on an hourly basis. After establishing the normal behaviour for a given species on an hour-to-hour basis, it has been necessary to resort, during subsequent work, to a 4-hourly recording. Even then these studies are very tiring to conduct. This work, being still in the early stages, can only be reported here in brief summary form.

The first species to be studied was T. fuscopennatus. Here oviposition was found to be exclusively nocturnal. It began just after dark, showed a short period of heightened activity in the period 19-20 hours (i.e., later than the main biting-peak). Thereafter activity remained at a low level, though gradually building up, till after midnight. The main oviposition period was in the last three hours before sunrise (Fig. 11). Oviposition did not follow rapidly after feeding, the recorded intervals varying from 5-16 days, with a median value of 7.

Another species which has been studied is A. africanus. A total of 210 females were fed and placed in a large cage, which was set up in a room at the Institute. The females were caught in forest and fed in small lots, the hour of feeding varying considerably, and the build-up of the colony occupied several days. During five days and nights of observation at hourly intervals 2,127 eggs were laid, 2,048 (96 per cent) by day. There was little activity in the morning, and most of the oviposition occurred in the afternoon. In the three-hour period 14-17 hours 944 eggs, 44 per cent of the total, were laid. The peak period was the hour 16-17, with 341 eggs. When geometric means are used, the fact that this hour was consistently the highest leads to a still more marked peak, as shown in Fig. 12. Thus in T. fuscopennatus both feeding and oviposition occur by night. In A. africanus feeding is crepuscular and nocturnal, whereas oviposition occurs by day. As the matter will be referred to again below, it is important to note that the peak period was the third 4-hour period after sunrise.

With these experiments showing a clear indication of periodicity, work was begun with another vector species, A. aegypti, which is easy to breed and handle in the laboratory and so is more suitable for detailed studies

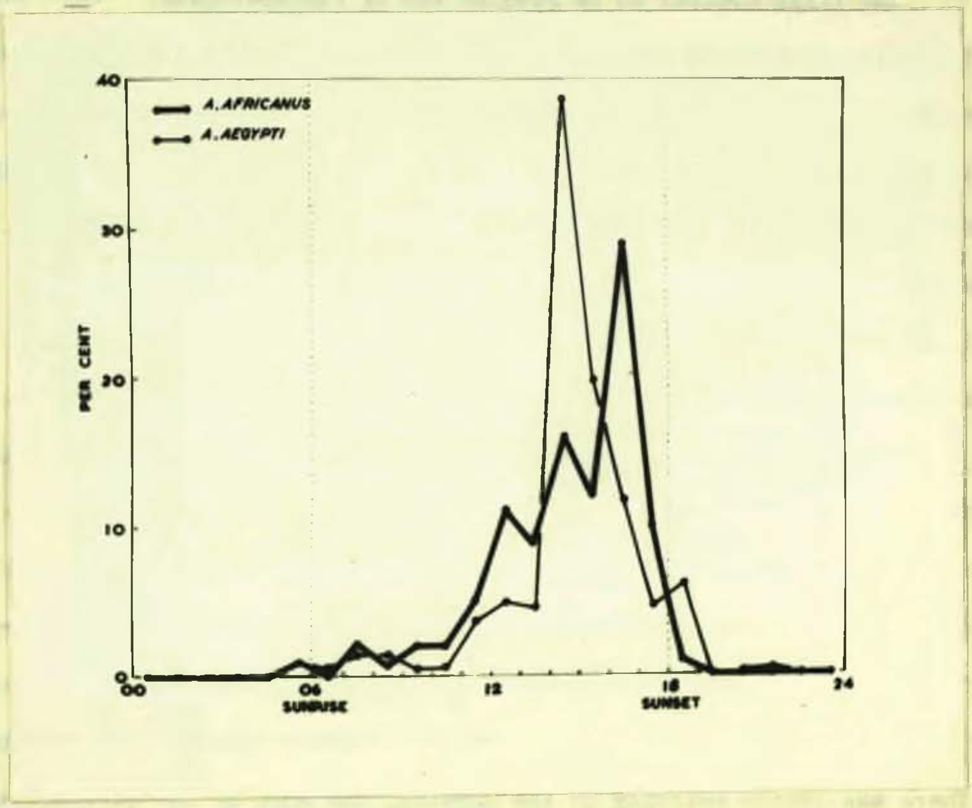


Fig. 12

The oviposition cycles of Aëdes africanus and Aëdes aegypti. The figures are geometric means of the numbers of eggs laid per hour, shown on a percentage basis to facilitate comparison.

than A. africanus. Two large cages were set up in a room at the Institute where normal daylight fluctuations take place without the entry of direct shafts of sunlight. Males and females of two strains (known to show certain differences in behaviour in other respects) were bred out, and each strain was allotted a cage. The final strength of each culture was about 1,000 females and a smaller number of males. Blood meals (rabbit) and sugar meals (raisin) were constantly present. Over five days and nights observation the two strains behaved so similarly that the results may be combined here. Altogether 1,922 eggs were laid during the observation period, almost all in the afternoon. The peak period, 14-15 hours, was a little earlier than that of A. africanus but the general trend was very similar (Fig. 12). The 4-hourly totals may be given here, together with the Geometric Means (M_G), and the latter in percentage form:

Period	22-02	02-06	06-10	10-14	14-18	18-22
Eggs laid	0	26	127	411	1133	225
M_G x 10	0	19	144	326	2060	137
M_G as %	0	1	5	12	77	5

Here appeared yet another variation of the pattern, a mosquito which feeds by day (Teesdale, 1955; Lumsden, in press) and which also lays by day. When the experiment was begun, controlled conditions were not thought of particularly. Records were, however, kept, and when it was found that in the cages the total temperature range had been only 2°C and the total range of relative humidity only 7%, it was concluded that light or some other component of solar radiation must be responsible for the periodic pattern, which was highly consistent from day to day.

It was decided that an attempt should now be made to break this rhythm by setting up colonies in constant microclimatic conditions. Two large cages were accordingly placed in a windowless controlled conditions room (hereafter "C.C." room) maintained at 27°C and 70% R.H. Light also was constant, being supplied by 4-foot fluorescent strip-lights, daylight type. Approximately 1,000 females and a smaller number of males of A. aegypti were now placed in each cage. The cultures both came from the same strain but, while the one had been reared in the insectary, where normal daylight fluctuations occur, the other had been reared from the egg in the C.C. room, in constant light. This was done to find whether perhaps a rhythm imposed during larval or pupal life, in the case of the culture reared in the insectary, might carry over into adult life. This did not prove to be the case and the results for the two cages may be combined. In five days and nights of observation at 4-hourly intervals, it was now found that oviposition was aperiodic. The sample was a good one, 9,414 eggs being recorded. The distribution in time was as follows:

Period	22-02	02-06	06-10	10-14	14-18	18-22
Eggs laid	1989	1239	2008	1236	1578	1364
M_G x 10	304	153	281	198	260	205
M_G as %	22	11	20	14	19	15

The next experiment was also conducted in the C.C. room, the object being to try to induce a periodicity by switching the lights off and on for alternating 12-hour periods - roughly corresponding to the duration of day and night at the equator, where Entebbe lies. A new large culture was used, and once more about 1,000 females were used in the build-up of the colony. In the five days and nights of observation at 4-hour intervals, 11,028 eggs were laid. It was found that laying now became markedly periodic, following the same pattern as that seen in the first experiment with this species and also in the experiment with A. africanus. That is to say, the main laying period was the third 4-hour period after the first appearance of light. Here, for reasons noted below, the periods are listed as "a", "b", etc. The results were:

Period	a	b	c	d	e	f
Light	off	off	on	on	on	off
Eggs laid	286	12	640	777	6822	2491
M_G x 10	94	7	869	604	13290	4540
M_G as %	< 1	<1	5	3	69	23

The pattern, it may be noted, was highly consistent and repetitive from day to day. The difference was that here, to eliminate any possible outside influence, day and night had been artificially reversed - i.e., the lights were switched on at 18 hours and off at 06 hours. The peak laying period was thus 02-06 hours. This experiment showed that light, and not another component of solar radiation, was the master factor, and that the oviposition cycle could be altered by altering the hours of light and darkness. No question of changing crepuscular light or of altering wavelengths could arise.

As it was known that the time between the blood meal and oviposition is roughly constant at a given temperature, it was necessary to repeat some of the foregoing work with insects all of the same age and all fed at known times, in order to eliminate any possibility that the rhythms described above were influenced by this fact. Three "inside" cages were set up in the C.C. room in constant light, and three "outside" cages in the room where the first experiment had been conducted - i.e., in a room with fluctuating light but with very constant temperature and humidity. In each cage 20 females and 10 males were placed, all these insects having emerged on the same day. Two days were allowed for mating. At 08 hours on the third day Gillett fed the insects in the first inside cage on his hand, the writer simultaneously feeding those in the first outside cage. Feeding was complete - except for three females - within the time allowed (10 minutes). Eight hours later, at 16 hours, this process was repeated exactly with the second pair of cages. Eight hours later still, at midnight, it was repeated with the third pair. Thereafter hourly records were kept. In the C.C. room eggs appeared in the first cage 58 hours after the blood meal, in the second cage 5 hours later, and in the third cage 5 hours later still. There was thus some "telescoping" of the intervals - eight hours between feedings being reduced to five hours between first oviposition. Each cage was watched hourly from the first oviposition for 48 hours and in all the laying was aperiodic, this result confirming those of the previous experiment in constant light.

In the case of the outside cages the onset of oviposition was later, as the ambient temperature (about 23^{o}C) was lower than that in the C.C. room (27^{O}C). Here eggs appeared in the first cage 77 hours after the feed, the actual time being the period 12-13 hours. Thereafter laying in this cage became normally periodic, as in the first experiment, with a peak in the afternoon and very little laying after dark. The second cage began laying 4 hours later than the first, at 16-17 hours, after which laying became periodic as in cage 1. Night fell shortly after this, at 18 hours. It may be deduced that the third cage should have become mature about 4-5 hours after the second, i.e., that oviposition should have begun in the period 20-21 or 21-22 hours. This did not, however, occur, and no eggs were laid throughout the night. It was concluded that the onset of oviposition had been inhibited by darkness, and that it would not begin till light returned. Oviposition did not begin with the first light, and actually no eggs were laid till midday (18 hours after the second cage), after which the normal periodicity with afternoon peak developed. Thus, it was concluded, a certain stage of ovarian development must be reached before light becomes important, and if this stage is reached at night, the onset of oviposition will be delayed (in another experiment it has been shown that in constant darkness the onset of oviposition may be delayed 20-30 hours as compared with the onset in a control group in constant light). A still more significant finding was that oviposition does not begin immediately after light returns. Instead, it begins after considerable delay and reaches its peak at the usual time, namely, in the third 4-hour light period. This suggests that when a certain ovarian stage has been reached a light-activated hormone comes into play. Preliminary experiments in the C.C. room - not yet far enough advanced for a detailed report - support this view. Even a short exposure to light will impose a pattern in which maximum oviposition occurs a fixed time after the first appearance of the light.

Strangely enough, a cyclical pattern also appears in mosquitoes kept constantly in the dark. Here, however, there is some evidence that a different mechanism may be responsible, and that hunger cycles may lead in turn to cyclical bursts of egg-laying. Work on this aspect continues.

A great deal of work lies ahead in order to determine critical lengths of exposure to light, critical light intensities, etc. Other mosquito species must also be studied. It is felt, however, that the effort will be worth while as the experiments discussed above are among the very few where it has been possible to link an important cyclical activity to a single microclimatic factor. It is for this reason that, though far from complete, they have been discussed at some length.

It is only by detailed and continued work, carried out personally or with the closest personal supervision, and with a maximum of actual observation that it will be possible to elucidate the numerous activities of mosquitoes - feeding, oviposition, mating, flight cycles, etc. - which must be thoroughly understood before we can form that complete picture of vector activity which will lead to adequate understanding of the transmission not only of yellow fever but also of other mosquito-borne diseases.

The Entebbe Tree Survey (and much of the other work discussed in this section) was essentially investigative, but selected groups of mosquitoes were inoculated into laboratory animals, mainly in the hope that yellow fever virus might be isolated, and routine catches were also conducted in the area for the same purpose, mainly in the small forests at Zika and Lunyo, near Entebbe. Unfortunately yellow fever virus has not yet been obtained, in spite of the fact that the number of Aëdes africanus inoculated is now considerable. Various other agents have, however, been isolated, as follows:

In June, 1946, a virus which was thought to be new was obtained. It was named Mengo encephalomyelitis virus, but has since proved to be a strain of encephalomyocarditis virus (EMC). As the virus is important, and as a good deal of work has been done on it, a short note on it is given in Appendix 7.

In 1947 a new virus named Zika virus was isolated in the Zika forest area, and this and subsequent isolations of the same agent are discussed briefly in Appendix 8.

At the time when these isolations were made, the standard procedure was the inoculation of adult mice. More recently, however, it has been found that infant mice are much more susceptible, and now a much greater number of virus strains can be isolated with less work in the field. Thus in the twelve months prior to the time of writing, 35 strains of virus, including 8 of a virus which is probably new, have been isolated by M. P. Weinbren, the writer and M. C. Williams at Entebbe. These have come from human cases (7), from domestic animals (17), from captive monkeys (5), from ticks (1) and from mosquitoes (5). Of the mosquito isolations 1 was from Aëdes circumluteolus and 4 were from A. africanus.

The virus isolation programme has tended to stress the great importance of A. africanus as a virus vector. Thus in the original isolation of yellow fever virus from forest mosquitoes in 1944 A. africanus was included in the infected lot and was in all probability the infected species. At the end of 1947 a laboratory rhesus died of yellow fever following inoculation of a suspension of A. africanus. In 1948 four lots infected with yellow fever virus were obtained and in the case of one of these lots a transmission was also secured by bite. In the work at Entebbe Zika virus was obtained from this species in 1947, and twice again in 1956. Finally, Rift Valley fever virus and Chikungunya virus were isolated once each during 1956 from A. africanus. More detailed notes will be found in the appropriate appendices, that on Chikungunya virus being included in the appendix on Semliki Forest virus and allied agents. Work is, therefore, tending to concentrate increasingly on this species. It is a highly specialised mosquito and, while much is now known of its biting habits, its oviposition cycle, its breeding places in tree-holes and its seasonal incidence, considerable gaps in our knowledge remain. Nothing is as yet known of its mating habits, and it has not yet been successfully colonised in the laboratory. The males have never been found in nature and their resting places must be sought for - probably in the forest canopy. The vitally important question of longevity has not been studied at all. Food preferences are not adequately known though it is believed that monkeys are a preferred host. Unfortunately almost all the resting females taken in nature are unfed, and the resting places of blood-fed females must be found so that precipitin test techniques may be used in the identification of the blood meals. Thus a great deal of work on this interesting mosquito lies ahead, and obviously some years must pass before an adequate picture of the natural history of A. africanus can be built up.

Certain other sections of the writer's work on mosquitoes are more appropriately discussed in the following sections, as they are essentially concerned with surveys of the vector species.

SURVEY AND EPIDEMIOLOGY IN THE WETTER FORESTED AREAS

The decision to establish a field station in Bwamba for intensive work was based on the fact that this area showed by far the highest incidence of immune Africans in the preliminary surveys carried out by Mahaffy and his co-workers (Hughes, Jacobs and Burke, 1941; Mahaffy, Smithburn, Jacobs and Gillett, 1942; Mahaffy, Smithburn and Hughes, 1946). These surveys did, however, reveal a number of immune individuals in various other Uganda localities. Some areas, indeed, in the West Nile and Acholi Districts and in the islands of Lake Victoria, showed a fairly high incidence of immunity, though never approaching that found in the Bwamba lowlands.

Findlay and MacCallum (1937) had published the results of tests on 20 grey monkeys (Cercopithecus aethiops centralis) from unrecorded Uganda localities, five of these showing antibody to yellow fever virus. Subsequently, in 1938, Dr. J. J. Paul, a member of Mahaffy's team, purchased a sample of grey monkeys from native trappers at Kiryandongo in Bunyoro District, and immune specimens were again found. Small samples from other parts of Uganda (Kome Island and Entebbe) did not at that time show any immune specimens. Finally, as mentioned above, Dr. H. R. Jacobs obtained a sample of five redtail monkeys (Cercopithecus ascanius schmidti) in Bwamba during 1939, and one of these was found to be immune. These monkeys were collected essentially for experimental laboratory work, and the discovery of immune specimens among them was incidental. Oddly enough these observations of immunity received hardly any attention at the time, the probable reason being that the main effort was being devoted to the search for a human case, as thus only could the existence of yellow fever in Uganda be proved to the satisfaction of the local authorities. Thus, for example, in the paper describing the first isolations of virus from a human case and from mosquitoes in Bwamba (Mahaffy, Smithburn, Jacobs and Gillett, loc. cit.) the occurrence of an immune monkey in a small Bwamba sample is not even mentioned in the otherwise very complete discussion of the situation. Even when the great yellow fever epidemic broke out in the Nuba Mountains in the Sudan (Kirk, 1941) attention was centred entirely on the human cases, and no monkeys were collected in that area till much later.

With the collection of the writer's sample of monkeys in Bwamba in 1942, however, attention became concentrated on this group more particularly as tests on other animals were all negative, and as the human population had been eliminated from the epidemiological picture by the recent mass vaccination. In the small sample obtained that year more than half the monkeys were immune, and included in it were three colobus and two mangabeys - monkeys which never leave the main forest - all of which were immune.

A further advance was made in 1943. Two redtail monkeys were captured by an African in the Entebbe neighbourhood and sold to the Institute. When tested, both were found to be immune. This was an unexpected finding, as tests had already been made on sera from 50 local Africans, and all had proved to be non-immune. Even when a wider area was considered, and the results for the whole of Mengo district (in which Entebbe lies centrally) were combined, it was found that there was only one immune specimen in the sample of 263 human sera, and this had been taken from an adult, who might have travelled. The discovery of immunity among the monkeys of an area where the incidence in man was so low as to be negligible was a finding of considerable importance, giving the first indication that virus might exist in what are now called "silent areas" without affecting the human population. Thus, where survey work was confined to man, misleading results might occur. Dr. T. P. Hughes now began to shoot monkeys in the Entebbe area, and his sample confirmed that immune monkeys were present in the wild populations of the area. After Hughes' departure from Uganda in 1943, this work was carried on by Dr. K. Goodner and the writer, though other members of the Institute staff also contributed some specimens. The results revealed that at that time the incidence of immunity among the monkeys of the lake-shore forests in Mengo district was very high indeed, reaching 79 per cent on Mbiru peninsula near Entebbe. Another area near Entebbe, Nakauka, gave an immune rate of 78 per cent in a sample of 36 monkeys collected by Goodner and the writer. It is interesting to note, however, that in the section of the Nakauka area covered by the writer the rate may have been still higher, as of the 14 monkeys he collected 13 were immune.

It was now felt that the original survey of immunity among the human populations of the various Uganda districts could not be relied on to give a true picture of the degree of virus activity in a given area nor, for that matter, of the geographical distribution of virus activity. It therefore seemed desirable to begin a survey of the forest areas of Uganda, sampling the monkey populations, and it was decided that, to be adequate, the sample from a given area should include not less than ten adult and old specimens. It was not, of course, always possible to obtain a sample of this size in the time available. The survey progressed slowly at first, owing to the necessity for intensive work in Bwamba, but by 1945 immune monkeys had been found by Hughes, Goodner and the writer in five areas in southern Uganda, and it was at that time decided to intensify the work as it was becoming obvious that immunity in monkeys must be very widespread in the Uganda lowlands. At the beginning of 1946 Dr. G. W. A. Dick joined the team, and he and the writer collaborated on this work, other members of the Institute staff contributing specimens from time to time. This survey also provided a good opportunity to study the distribution of A. simpsoni and A. africanus and to take new samples from the human populations. Dick's main work was to collect the human sera, but he contributed very substantially to the monkey samples also. The writer collected monkeys and mosquitoes. The mosquito collections were carried out as follows: Each day the main work was the collection of monkeys, but the team of African collectors worked in the plantations, confining their attention to the important plants, until a sample of larvae had been obtained from 1,000 water-containing axils in the locality concerned. The A. simpsoni survey was thus based on larva collections, the reason being that larvae are always very much more easily found than are the adults. In the case of

A. africanus, collections were made from 100 water-containing tree-holes, in case adults should not be found. Here, however, the main survey was based on adult catches and was carried out by making catches in trees - usually in swamp-forest - in the sunset period. The reason that this part of the work was based on adult catches was that it was considered desirable to establish that the biting habits of this mosquito were everywhere arboreal and crepuscular, and thus collections of biting females were necessary.

By the end of 1949 forty localities (if Bwamba be included) had been sampled. Unfortunately, as the initial interest had been centred on the forests, the area covered represented only about half of Uganda. The drier areas in the north (Teso, Lango, Acholi and Karamoja Districts) remained unsampled or almost so, though Lumsden and Buxton (1951) investigated the other northern area, the West Nile district. The results showed that A. simpsoni and A. africanus were present everywhere (except in the mountain forest of Ruwenzori and the mountain bamboo forests of the Kigezi highlands). Similarly, in every locality where an adequate monkey sample was obtained, immune monkeys were found, once again the Ruwenzori forest proving the exception. It is unfortunate that monkeys have not yet been obtained in the other mountain forests of Uganda, notably from Mount Elgon, the Birunga volcanoes and the Chuya bamboo forest in Kigezi. It seems unlikely, however, that immune specimens will be found when an opportunity for sampling does occur. For example, it is known, as mentioned above, that in the Birunga and Chuya forests A. africanus is replaced as an arboreal and crepuscular species by two other members of the subgenus Stegomyia, A. bambusae and A. angustus (Haddow and van Someren, 1950).

As the Institute monkey sample by this time included over 1,000 Uganda specimens, it was felt that a new review of the relationship between monkeys and yellow fever in Uganda was called for, the previous paper (Haddow, Smithburn, Mahaffy and Bugher, 1947) having been based on a relatively small sample (150 monkeys) and confined to Bwamba County. In the new paper (Haddow, Dick, Lumsden and Smithburn, 1951) the large sample and much wider area of study led to changes in some of the conclusions previously reached. This was mainly due to the building up of adequate numbers of species which had been poorly represented in the Bwamba series. An opportunity also presented itself for a new review of the Bwamba lowland material, the final sample being, as noted above, 277 monkeys.

It had become a matter of some importance to find whether the estimate of the duration of the various age grades was reasonably near the truth, and it was felt that with the present large sample some advance could be made in this matter. If for the purpose of argument it were assumed that all monkeys survived to reach adult status, then the numbers of monkeys in this and the younger age-grades would be proportional to the duration of the grades as, in most species, breeding seems to go on throughout the year. A theoretical frequency distribution was prepared on this basis and compared with the actual distribution of age-grades in the sample. It was found that the fit was extraordinarily close, and thus there was nothing to suggest that the duration of the age-grades, as estimated, was incorrect (it is now known from direct - unpublished - observations that in at least some species the estimate is approximately correct). The other conclusion, at first sight a surprising one, was that perhaps a very high percentage of monkeys do survive to attain adult life. On further reflection the conclusion seemed less surprising as, though monkeys do not breed till the age of 5-6 years (at that time 3 years was thought to be the earliest breeding age) and though they reproduce slowly, not more than one offspring per mother per year being born, they are none the less one of the most flourishing mammal groups of the tropics. Further, their predatory enemies, apart from man, are remarkably few, and population-reducing epizootic diseases (such as rinderpest in ungulates or distemper in canines) are not known to occur among them. This subject is discussed more fully in the paper mentioned above.

Analysis of the sample showed that about 42 per cent of the monkeys tested were immune. If the negative Ruwenzori area was omitted the figure rose to 44 per cent. While the incidence varied greatly from district to district, the occurrence of the infection was obviously very widespread. Bwamba County and certain areas near the shores of Lake

Victoria in Mengo and Masaka districts showed the highest incidence of immunity.

Five species were represented in sufficient numbers to permit individual discussion. These were the black mangabey (C. albigena johnstoni), the redtail (C. ascanius schmidti), the grey monkey or grivet (C. aethiops centralis), the blue monkey (C. mitis stuhlmanni) and the lowland colobus (C. abyssinicus ituricus). It was found that in the grey monkey the incidence of immunity was significantly lower than in the others, there being less than one chance in 100 that differences of the order observed could arise fortuitously. The grey monkey is largely terrestrial by day, whereas the mangabey and colobus are strictly arboreal and the redtail and blue monkey very largely so, though they will descend for brief periods to attack crops. It was felt that perhaps the variation in rates as between different areas might have contributed to this unexpected result, but in the sample from one particular area there were enough grey monkeys and redtails to permit a direct comparison of immunity rates within a fairly small stretch of country. Here, unfortunately, part of the sample was of unknown age. The results were as follows: In 143 redtails the crude immunity rate was 77 per cent, while among 80 grey monkeys it was only 41 per cent. When the sample was confined to specimens of known age-grade and was standardised for age distribution, the immunity rate was 63 per cent among 52 redtails, and only 18 per cent among 16 grey monkeys.

Such being the case, it seemed worth while to sub-divide the entire Uganda lowland sample into habitat groups - arboreal, mainly arboreal and partly terrestrial. There was no significant difference between the first two groups, but in the partly terrestrial group the immunity rate was significantly lower than in the other two, the chances that the observed differences were fortuitous once again being less than one in 100. Now as all monkeys sleep in the trees by night, when A. africanus is active, all species should show a similar incidence of immunity, provided that this mosquito is the only vector. As, however, the partly terrestrial species showed a lower rate than the other groups, the question arose as to whether there might be a so far unknown arboreal vector, active by day. It should be mentioned that the more arboreal monkeys feed actively in the morning, but take to the canopy well before midday where they rest, and even sleep. Active feeding does not begin again till late afternoon and continues almost till sunset - this is discussed in detail elsewhere (Haddow, 1952a). If transmission occurs among monkeys by day, then they would be most vulnerable during the midday resting period. At this time the two most active arboreal mosquitoes are A. apicoargenteus and A. longipalpis. The former is, however, mainly a mosquito of the under-storey, and it is unable to transmit yellow fever virus by bite. A. longipalpis, on the other hand, bites mainly in the canopy, where the monkeys rest. Unfortunately, its capacity to transmit has not yet been tested. It is known, however, that it will bite monkeys in nature (Haddow and Dick, 1948). Further, while it is usually very difficult to feed and maintain in captivity, it will feed avidly on rhesus monkeys in the laboratory (J. D. Gillett, private communication). Further work on this interesting mosquito is contemplated by Gillett.

The large Bwamba sample was particularly suitable for the study of possible differences between the immunity rates in different environments within a single restricted area. It was now divided into three groups of monkeys - those from the forest interior, those from the forest edge, and those from the mixed forest and agricultural land of the inhabited area. It was found that there were no significant differences. On the other hand, outside Bwamba, considerable differences were found between different forests - even when these might be quite close to one another. Thus the Kibale and Itwara Forests in Toro are separated by only about 10 miles of grassland and bush, yet the standardised immunity rate for Kibale Forest was 45 per cent, while that for Itwara Forest was only 6 per cent. Confining the discussion to the colobus, it was found that there were no immune specimens in the Itwara sample, while in Kibale the standardised rate was 58 per cent. A similar state of affairs occurs in Bunyoro District, where the sample from Budongo Forest showed a standardised immunity rate of 40 per cent, whereas in the Bugoma Forest, only about 25 miles away, it was only 21 per cent. The reasons for these marked differences were not revealed by the mosquito surveys, and have remained obscure till the present.

Similarly, the incidence of immunity may be patchy within a small area. Thus ten small separate patches of forest on the Entebbe peninsula were sampled, the general immunity rate in the sample of 66 monkeys being 45 per cent. Immune specimens were found in nine of the ten areas. The tenth, from which seven monkeys were obtained, yielded no immune specimens. Once again the reason is not understood. The forests concerned are all grouped within a very small area, and A. africanus is abundant in all of them.

When the age-distribution of immune monkeys was considered, the same picture was obtained as before, namely, an "enzootic curve" showing steadily rising incidence with increasing age (once the infant grade was passed). Infants are born to mothers in the adult and old age-grades. In these grades (combined) the incidence of immunity was found to be 60 per cent. Thus it may be presumed that about 60 per cent of newborn Uganda monkeys possess the transient passive immunity passed on to them by the mother. The percentage incidence of immunity by age-grades was now found to be:

Age-grade	Per cent immune
At birth (theoretical)	60
Infant (observed)	25
Juvenile "	2
Subadult "	18
Adult "	55
Old "	81

When the Bwamba lowland sample - the largest from a single area- was considered by itself, the general trend was found to be very similar, the figures being:

Age-grade	Per cent immune
At birth (theoretical)	77
Infant (observed)	31
Juvenile "	0
Subadult "	14
Adult "	72
Old "	91

When the figures for the more abundant species were extracted for individual attention, it was found that in the case of the redtail monkey the form of the curve differed from the above type, as exemplified by the lowland colobus. Thus in the colobus immunity declined till the juvenile age-grade was reached, after which it increased gradually, as above. In the redtail, on the other hand, the incidence of immunity at first decreased, as in the colobus, but in the subadult age-grade there was an abrupt rise, though by the time that full adult life had been attained the two species once more showed a similar incidence of immunity. The old age-grade in the colobus showed a higher incidence than the corresponding grade in the redtail. Perhaps (though this is merely conjecture) these large monkeys may survive longer in this grade than does the small redtail. The actual figures were as follows:

Age-grade	Per cent immune	
	Redtail	Colobus
At birth (theoretical)	62	69
Infant (observed)	33	29
Juvenile "	0	5
Subadult "	43	11
Adult "	61	64
Old "	65	87

At the time when the work was written up, this difference appeared puzzling. The probable explanation was found some time later, when analysis of the records revealed that in the redtail there appears to be a breeding season. Thus females in early pregnancy were obtained only between January and May, females in advanced pregnancy only between May and September, and females with young infants only between September and April. In the great majority of monkeys there is no defined breeding season (Zuckerman, 1932). In the colobus sample, for instance, the evidence clearly pointed to continuous breeding throughout the year, females in advanced pregnancy being present at all times (Haddow, 1952a). It is a commonplace of epidemiology that, when a large number of non-immune individuals enter a population at a given time, conditions become more favourable for the rapid spread of an infection from individual to individual than is the case when non-immunes enter the population gradually, over a more extended period. Thus in the redtail the addition to the population of a considerable number of non-immune juveniles over a limited season could create conditions under which the chances of immunisation at an early age would be enhanced, and this is thought to be the reason for the sudden rise in the incidence of immunity in the subadult grade. In the colobus, on the other hand, the more constant addition of a smaller number of immunes throughout the year would lead to conditions under which the passage of the infection from monkey to monkey would proceed more continuously, but less actively. There is, of course, no theoretical reason why the final incidence in adult life should differ. This subject has been discussed more fully elsewhere (Haddow, 1952a).

Broadly speaking, the general picture suggested either a continuous enzootic, or an enzootic in the form of a series of frequently-repeated waves. In Bwamba, for example, the incidence of immunity might rise or fall, but it was at all times very high. Between July, 1942, and December, 1947, the immunity rate for any given six-month period was always over 40 per cent. There was, however, evidence that more active outbreaks may occur among monkeys in Uganda, as follows:

A large sample of grey monkeys was purchased by the Institute from native trappers on Bukasa Island in the Sese Archipelago, Lake Victoria, during April, 1943. Of 33 monkeys, only one was immune, the immunity rate thus being 3 per cent. Nine months later, in December, 1943, a further collection of 36 monkeys from the same island was purchased. Of these 32 were immune, the rate thus being 89 per cent. Here there was clear evidence of epizootic yellow fever among the monkeys of Bukasa. Equally interesting was the course of events on Kome Island, a member of an entirely different group, not far from the Entebbe peninsula, but separated from the Sese Islands by about 20 miles of open water. The monkeys from this island, collected by African trappers, by the writer, and by other members of the Institute staff showed the following immunity rates in the various samples:

April, 1940	0/13)		April, 1944	6/23)	
May, 1941	0/ 1)	0/53 - 0 per cent	May, 1944	1/ 8)	9/42 - 21 per cent
March, 1943	0/39)		March, 1949	2/11)	

Thus there was no evidence of monkey yellow fever on Kome till 1944, since when the infection has apparently remained active there. It appears that the change occurred at about the same time as the Bukasa epizootic, as is shown by the following figures:

Island	Date	Per cent immune
Bukasa	April, 1943	3
Kome	March, 1943	0
Bukasa	December, 1943	89
Kome	April, 1944	26

The situation in these islands will be discussed further below. Another example of intense virus activity within a limited area was provided by the outbreak in Bwamba during 1948, with a high incidence of infection among the sentinel monkeys. It is to be emphasized, of course, that an epizootic among African monkeys will not present the dramatic picture seen in the New World, as almost all will recover after a mild fever. It is unlikely, in fact, that anything unusual will be noticed by the local people.

A puzzling feature of all this work was that over most of Uganda, no matter how high the incidence of immunity among the local monkeys might be, there were very few places where the incidence in man was high, and none which showed figures in any way comparable to those found in Bwamba. Various explanations were put forward. For example, following the demonstration that there had been an epizootic among the Bukasa monkeys, T. P. Hughes sampled the human population (which had previously been sampled in 1943). The incidence of immunity in 1943 was 12 per cent in a sample of 110 sera, and in 1944 it was 11 per cent in a sample of 66 sera. Thus obviously there had been no human epidemic corresponding to the monkey epizootic. During a subsequent visit to Bukasa the writer concluded that the reason might be the compulsory segregation of the people away from the waterside forests (a measure to reduce the incidence of human trypanosomiasis). In Sese generally the forest borders the shore, and inland the islands are open and grassy. In Mengo District, however, and particularly around Entebbe, there was a high incidence of yellow fever immunity among the monkeys, and A. africanus is always abundant. Plantations are in close contiguity with the lake-shore forests, and are continually being raided by monkeys. At the same time, the incidence of immunity in man in this entire area is so low as to be negligible. The favourite breeding-plants for A. simpsoni, the gonja banana, colocasia and pineapple, are not very abundant, but at best this seemed a very inadequate explanation of the above situation. While reading the typescript of the paper concerned (Haddow, Dick, Lumsden and Smithburn, loc. cit.), Gillett suggested what is probably the true explanation. While discussing the problem with the writer, he remarked that he could not remember ever having been bitten by A. simpsoni at Entebbe. This was a most important observation, as Gillett had had experience of the area at a time when A. simpsoni was exceedingly common there, and had worked intensively in the banana plantations during the campaign against this species which took place after its incrimination as a vector in Bwamba. As the survey of A. simpsoni discussed above had been based on collections of larvae, the question of whether human blood was always acceptable to this species had not arisen, as it almost certainly must have done had adult catches been the basis of the work. Acting on this lead, Gillett now carried out a good deal of adult catching, including 24-hour catches, in a number of Uganda localities. It soon became obvious that A. simpsoni exists in populations which bite man and others which do not and further, that the areas in Uganda where human blood is taken are relatively few. Gillett at first thought that altitude played a part in determining this difference but this view later proved to be inadmissible. The explanation of these behaviour differences is not yet known, but it may have a genetic basis (Gillett, 1951a; 1955). It will be remembered that some years before it had been shown that A. africanus may infiltrate plantations in small numbers by night (Haddow, 1945b) and Gillett, who also found small numbers of this species in plantations, now suggested that these stray mosquitoes might possibly account for the scattered immune individuals found in areas where A. simpsoni does not appear to bite man. It may be mentioned that there is as yet no information as to the preferred food of A. simpsoni in such areas.

As has been mentioned above, the collection of blood samples from wild monkeys gave an opportunity for recording weights, measurements and other biological data, in addition to which a considerable amount of time was spent from 1942 onward in the observation of monkeys in their natural habitat. When the paper discussed above was completed it was felt that future sampling would be more limited and localised, and devoted to particular groups. Wide general survey in Uganda could no longer be carried on, partly on account of the rapidly rising cost of field work. It seemed appropriate, therefore, to analyse and write up the zoological and other data which had been collected on monkeys. Only three species - the redtail monkey, the lowland colobus and the black mangabey were sufficiently well represented in the writer's sample to be worthy of individual attention. The plan was to produce three papers, one on each of these, and to follow up with less detailed studies on the other Uganda monkeys. Owing to pressure of other work, only the first of these papers, that on the redtail, has so far been published. This monkey was selected for prior treatment in view of its importance in relation to yellow fever, particularly where the conveyance of virus from forest to plantations is concerned. Apart from this aspect the monkey has another importance, as it is classed as a pest in agricultural areas. The paper concerned (Haddow, 1952a) is of considerable length and cannot be reviewed adequately here. In view of the fact that the wild animals of Africa are rapidly being exterminated over enormous tracts of country and as there is remarkably little available in the way of detailed studies on individual species, an effort was made to give as complete a picture as possible of this single animal, and it is gratifying to find that the attempt has been considered successful by at least one authority (Harrison Matthews, 1954).

While in the case of the larger rain-forests the continuous passage of yellow fever from monkey to monkey through the agency of an arboreal mosquito vector seemed an adequate explanation of the situation, as some of these forests have monkey populations of 50-100 per square mile, a puzzling state of affairs was noted in the case of some of the small patches of forest which were sampled during the general survey. A small forest - say one to two square miles in extent - might be isolated from the nearest large forest area by many miles of open grassland or scrub, with no monkeys and no sylvan mosquitoes. The total monkey population of such a forest patch might be only 30 or 40 individuals. Yet, on the collection of a sample, the familiar pattern of immunity in relation to age would be found. In addition, evidence of yellow fever was to be found in almost all of the small forest patches sampled. The position was thus that the virus was active over a very wide area, often in small patches of forest and in these, as in the largest forests, the evidence available suggested that the infection was enzootic. The anomaly was that in many of these small forests the monkey population was inadequate to support an enzootic disease. As no reservoir - apart from the mosquito host - was known, it was very difficult to see how the infection maintained itself, more particularly in the numerous areas where the occurrence of a marked dry season periodically reduced the numbers of adult mosquitoes to a small fraction of the wet-season population.

In this connection two possible explanations presented themselves. The first of these will now be discussed. The second forms the basis of the next section.

It was felt that virus might be continuously active in the large rain-forests and that seasonally - probably during the rains - it might be disseminated over wide tracts of country by mosquitoes. Various workers have shown that small insects may be carried to great heights by convection currents, and presumably may then be dispersed over long distances by wind. In this field the studies of Johnson on aphids are particularly important (see, for example, Johnson, 1953). A particularly interesting example of wide dispersion of a mosquito in Africa is that given by Garret-Jones (1950). The whole subject of insect flight range has recently been discussed by Hocking (1953). In his summary of published information he quotes 23 cases of mosquitoes travelling distances over 20 kilometres. In seven of these the distance was over 50 Km. and in 3 it was over 100 Km. In this connection it is interesting to note that Wellington (1945b) has pointed out that thermal convection currents occur over forest in the sunset period. It is at this time that swarming is most frequently noted (Nielsen and Greve, 1950), and the same workers have shown that there is at this time a tendency for mosquitoes to rise and to form high-flying swarms over trees. Should this occur over the rain-forest canopy, conditions might be ideal for the carriage of mosquitoes to considerable heights by thermal convection currents and for their subsequent dispersal by wind over wide areas. It is perhaps significant that the writer has never taken the males of any of the really arboreal species such as A. africanus and A. longipalpis in nature. They presumably rest in the canopy by day and perhaps they are active over it by night. Plans have been made for an investigation of the activities of mosquitoes above the canopy, but have for several years been frustrated by the exceedingly high cost of the necessary apparatus - the most important item being a tower of steel scaffolding 120 feet high with a platform on top (well above the canopy), one at 80 feet (in the canopy) and one at 40 feet (in the understorey). There is every hope that such a tower will be built within the next year and that the question may then be investigated in some detail. Until baited catches and trap catches with and without light can be carried out well above the canopy it will not be possible to appraise finally the likelihood of mosquitoes being dispersed in the manner just discussed.

Dispersal of mosquitoes over long distances would provide a simple and attractive explanation of many of the known facts. In Uganda there may already be some support for this suggestion in that the monkeys of Bukasa Island suddenly underwent an epizootic, as described above, while on Kome Island (previously negative) a high incidence of immunity appeared in the same year, the evidence suggesting that virus had suddenly been introduced from outside. In this connection it is of interest to note that in recent years the incidence of immunity has again declined. Thus, of 18 monkeys received from Bukasa Island during the last three years, only one has shown immunity to yellow fever virus, and conditions appear to be favourable for another epizootic. This position is being watched with interest, more particularly as monkey samples from the adjoining mainland in Masaka and Mengo Districts have also shown a much lowered incidence of immunity - it will be remembered that some parts of this area showed the highest immunity rates of all during the original monkey survey. It may be that infection is at present proceeding very slowly, or possibly it may not be occurring at all, in which case the arrival of infected mosquitoes from a distant focus might well initiate a new and very pronounced cycle of virus activity.

The marked fluctuations in immunity rates in central Uganda are, of course, in strong contrast to the position in Bwamba, where the rate remained high over a period of more than six years. Bwamba is itself, of course, a major forest area, with an enormous Primate population, and in addition, the Semliki Forest is continuous with the Ituri Forest of the Congo Basin - one of the world's largest rain-forest areas. Thus, in all probability, infection is always present in one part or another of the Semliki Valley.

Though much of the picture in the drier areas may be explained as being due to periodic reintroductions of virus from distant foci by mosquitoes, this theory fails to explain all the known facts, and in some areas at least it has been concluded that another mechanism must be at work. This matter is discussed more fully in the next section.

STUDIES ON THE YELLOW FEVER SITUATION IN THE DRIER AREAS

The early surveys of immunity in man in eastern Africa showed that immune individuals occur in many dry or seasonally dry areas, and the great epidemic in the Nuba Mountains in 1940 occurred in country which may be described as semi-desert. Since then there have been sporadic cases in man in East and Central Africa, sometimes in relatively dry country, or at least in country without rain-forest.

The first of these to be recorded was a fatal infection in an African at Kitale, Kenya, in 1942. This case was investigated by Mahaffy and Hughes (Mahaffy, 1949), but no explanation of its occurrence was found. Kitale lies in open farm country at an altitude of nearly 6,000 feet and, in spite of quite extensive survey, no local sign of the disease was discovered. Altogether 12 monkeys were shot in localities near Kitale

(Endebess, Soy and Cherangani) but all were negative. Further, a sample of 166 human sera from the same neighbourhood (Kitale, Endebess and Kapenguria) revealed no sign of infection in man. Nevertheless, the patient in this case had been actually in Kitale township for some months before her death.

A few months later another fatal case occurred, this time in a European missionary at Torit, in the Southern Sudan. Mahaffy studied the area and, though he found nothing suggestive in the immediate neighbourhood of Torit, he did find a high incidence of immunity in the inhabitants of the Imatong Mountains, which rise like a forested island from the dry plains of the Uganda-Sudan border. The Imatong range is 30-40 miles south of Torit, but there is considerable lorry traffic between them (Mahaffy, Smithburn and Hughes, 1946). There may have been, in the writer's opinion, a connection between these two cases as, at the time, considerable military convoys were passing through Kitale, heading to or from the Abyssinian front, either via Lodwar and Lokitaung in the North Frontier Province of Kenya, or via Torit and the Southern Sudan.

The next case was also fatal, the patient being an African soldier who died at Kisumu in Kenya. It was, however, established that he must have acquired his infection before arriving there and that in fact he must have become infected in the Langata Forest near Nairobi. Considerable interest attached to this case, which was investigated by Mahaffy and the writer. As it was some time before the patients' movements could be traced, the writer began work in the Kisumu area, with which he was already familiar, while later - when the man's movements had been worked out - Mahaffy began work at Nairobi. Investigations in the Kisumu area included the collection of monkey sera in the Kaimosi Forest, a large rain-forest area with distinct western affinities, lying at a height of about 5,000 feet. Garnham and Harper, who were at that time working in the area, took part in this collection. In the Kaimosi-Kakamega area 12 monkeys were collected, but all were non-immune. Subsequent studies in the Kaimosi Forest by Garnham, Harper and Highton (1946) revealed that both _A. africanus_ and a wild population of _A. aegypti_ occur in this forest, though _A. simpsoni_ is not known in the area. Apparently, however, virus is not active there nor, indeed, in the whole of the Nyanza Province, within which Kaimosi lies. Thus, between 1940 and 1943 (the time of the study under discussion) 338 human sera had been collected in the Province, all being non-immune. As Sawyer and Whitman (1936) found an immune African at Kisii, South Kavirondo District, Nyanza Province, the area has always been watched with interest. The collection of a large monkey sample, however, by Mr. J. C. McMahon, of the Kenya Medical Service, has not revealed any immune specimens, though 40 from this area (all C. aethiops johnstoni) have now been tested at Entebbe (Haddow, 1952c; Haddow, Lumsden, Hewitt and Mason, 1956).

On the other hand, the work initiated by Mahaffy in the Nairobi area did finally show evidence of virus activity there, though only after a prolonged search. Thus, of 166 monkeys tested, only 2 were immune. Both of these were included in a sample of 54 specimens from the Ndeya Forest, which is continuous with the Langata Forest. Mahaffy's sample of 60 human sera from the area also included three immune individuals, one of these three being a child, and none having moved from the area since birth. An analysis by Lumsden (1954) suggests that other immune humans later found in the same area may have been vaccinated (the final number stands at 20 immune of 175 tested, or 11 per cent). Lumsden tends to cast doubt - on theoretical grounds - on the validity of the immune specimens found by Mahaffy. The writer feels that he would not have done so had he taken part in the investigation concerned. The case had the most serious political and quarantine implications and, while most of the monkeys were collected by the Kenya Medical Service, the human sample was collected personally by Mahaffy, one of the world's most experienced and gifted workers on yellow fever. After the discovery of the immune Africans the Kenya authorities conducted a searching investigation in the hope of having the findings set aside, but without success. The fact that this area has produced a fatal case of yellow fever and that two immune monkeys and several immune humans have been found there appears overwhelming evidence of a true local activity - as opposed to introduction - of the virus.

In the same year, 1943, evidence was obtained that yellow fever was active far to the south, in Northern Rhodesia. Previous samples from various areas there, totalling 122 sera, had shown no evidence of immunity. Now, however, a suspect case occurred at Balovale, near the Zambezi River. The man was found, after recovery, to be immune, though there was no final proof that the immunity had been acquired during that particular illness. This incident led, however, to a survey of the area and, of 176 sera tested 15 (8.5 per cent) were found to be immune. This unexpected finding led to extensive survey work in the area and by 1953 a total of 3,238 Northern Rhodesian sera, from 12 localities, had been tested. Seven of these localities showed the presence of immune people and in three, all from the same general area, the rate was high (Balovale, 12 per cent; Mongu, 18 per cent; Senenga, 11 per cent). Thus obviously yellow fever was active over a wide stretch of country, without appearing in epidemic or easily recognisable form, just as in East Africa. It may be mentioned in passing that immune individuals have been found even further to the south in Southern Rhodesia and Bechuanaland (Smithburn, Goodner, Dick, Kitchen and Ross, 1949). It is important to note that in a large sample of monkeys collected from the areas of high human incidence in Northern Rhodesia, none have been found immune (Dr. B. de Meillon, personal communication).

In East Africa the next case to be reported was a fatal infection in a European in Toro District, West Uganda. At first sight this appeared to be another "dry country" case, as the area where the infection occurred is hot short-grass country with orchard bush. Investigation on the spot, however, revealed that there was a small belt of forest in a river valley very near the camp where the infection took place. In this forest <u>A. africanus</u> was to be found even in dry weather, and specimens were also taken in the banana plantations which lie between the forest and the camp, extending almost to the latter. Immune monkeys were present among a small sample collected in the forest. In other words this case, reported in more detail by Ross, Haddow, Raper and Trowell (1953) appeared to be the usual type of forest edge infection, though it would certainly have been considered as dry-country yellow fever had the field investigation been omitted.

Among the above cases there were some which might have been due to transport of virus from a large forest area (as in the Kitale, Torit and Toro cases). On the other hand, in the Nairobi case and the Balovale suspect case (and in the instance of at least some of the immunes from the Balovale area) this did not seem at all likely to have occurred. There are, indeed, various small forests around Nairobi, but these dry up to a considerable extent seasonally, and it seems certain that there must be breaks in the maintenance of any virus disease where a mosquito breeding in small containers is the main reservoir. A very important additional point is that A. africanus and A. simpsoni do not occur near Nairobi. Garnham (1949) has shown that the commonest arboreal and crepuscular mosquito of the small forests of this area is A. deboeri, yet another member of the subgenus Stegomyia, whose capacity to act as a vector has not yet been tested. Garnham (loc. cit.) has also pointed out that A. aegypti occurs in these forests, breeding in tree-holes and artificial containers, and the writer on one occasion made a catch in which two mosquito boys took over 40 A. aegypti in the undergrowth during a single morning. The main facts which have emerged, however, are that in this area continuous maintenance of a virus infection by mosquitoes is improbable, and that while immunes do occur among the monkeys and local Africans, the immunity rate is very low.

From the earliest days of the investigations in Africa and in South America, the view has persistently come to the fore that there must be, in some areas at least, a host-virus cycle of which nothing was known. This view has been common to most of the workers in this field, in spite of the fact that until recently the evidence was almost entirely negative. In South America, of course, the part played by marsupials is still under discussion (see Bugher and Taylor, in Strode, 1951) but it does seem that these animals, with their comparatively large populations might well explain the situation in areas where simple monkey-to-monkey transmission seems an inadequate explanation of the epidemiology. In Africa, however, there is no suggestion that any mammal group apart from the Primates is involved in the maintenance of the virus. The importance of monkey-to-monkey passage is undoubted, and in many areas it is probable that mosquitoes carried long distances by convection and drift effects may also play a part in the periodic reintroduction of virus. In some districts, however, it seems unlikely, in view of the sparse distribution of monkeys, that stray infected mosquitoes introduced in this manner could effectively start an outbreak, even if they arrived at a season when local mosquitoes were common. In some such areas, however, there is clear evidence that the virus is active, as will be shown below. The question has been discussed in more detail elsewhere (Haddow, Dick, Lumsden and Smithburn, 1951).

Among suggestions that have come to the fore, the possibility of transovarial transmission of the virus from generation to generation in the arthropod vector is one that has seemed particularly attractive. This possibility was, however, studied and excluded in the case of A. aegypti by various of the earlier workers, the best summary of the results being that given by Davis and Shannon (1930). A negative result was also obtained in the case of A. africanus, which was thoroughly tested at Entebbe (Gillett, Ross, Dick, Haddow and Hewitt, 1950). It seems unlikely, in fact, that transovarial transmission of virus occurs in mosquitoes. It has, however, been shown that in sandfly fever the virus can pass from one generation of Phlebotomus to the next (Moshkovsky and six others, quoted by Rivers, 1952) and it will be remembered that an isolation of yellow fever virus was made from Phlebotomus spp. in Bwamba in 1948 (Smithburn, Haddow and Lumsden, 1949b). Syverton and Berry (1941) have shown that in ticks transovarial passage of the virus of western equine encephalomyelitis may occur. Russian workers (whose papers have been seen by the writer in review and summary form only) claim similar results with various tick species and the virus of Russian far eastern encephalitis. Up till the present yellow fever studies on ticks have produced conflicting results (Aragao, 1933; Davis, 1933). The second of these writers showed persistence of virus for varying periods, but obtained no transmission by bite and secured no evidence of transovarial transmission. Further work on this group is required, but it must be remembered that ticks are rarely found on wild primates.

At about the same time that immune monkeys were obtained in the Nairobi area the first definite suggestion of another cycle was obtained, this also providing a clue to the situation in the drier country. Samples of monkey serum had been sent to the Institute from the Coast Province, Kenya, for survey, and from time to time live galagos or bush-babies (Galago crassicaudatus lasiotis) had been sent for experimental work in the laboratory. These animals are Primates, members of the Lemuroidea, and quite closely allied to the true lemurs of Madagascar. In 1945 one of these galagos, newly received at Entebbe, was found to be immune. Subsequently other immune galagos were obtained from the same area and Smithburn (1949), working with animals sent up from the coast, demonstrated that this species is highly susceptible to the Asibi strain of yellow fever virus. The mortality rate among the galagos studied was (when deaths due to intercurrent disease and to cardiac puncture are excluded) over 50 per cent. Subsequently Dick (1952a) showed that the death rate might be much lower when an East African strain of virus was used, though all the animals tested circulated the agent freely, and later became immune.

The situation on the Kenya coast, where man is concerned, has never been clear as, immediately after the Nuba Mountains outbreak, the human population of the entire coast was vaccinated by the local authorities, without a previous sample having been taken to appraise the position. Since this original campaign, various smaller vaccination campaigns have been carried out, and at present it is obviously unsound to base any critical conclusions on the incidence of immunity in man within the coastal belt or within various other interesting areas in Kenya. Though the intention behind the original campaign on the coast was praiseworthy - the protection of India and the Far East against any possible spread of the disease from the Sudan epidemic - it must be a matter for lasting regret that a sample of human sera was not obtained by the local authorities before immunisation was begun. Where animals were concerned, however, information continued to accumulate and, after some years' work, several immune galagos had been obtained. Monkeys were also collected on the coast (by members of the Kenya Medical Department) and among these were two immune specimens. Where monkeys were concerned, the situation resembled that in the Nairobi area, the percentage of immune specimens being very low.

At about this time a survey of the yellow fever situation in the rather dry country of the West Nile District, Uganda, was made by Lumsden and Buxton (1951). Immune monkeys were found, but the sample of galagos obtained was small - only three - and none showed immunity. Here the species concerned was the small galago of the open bush country, G. senegalensis senegalensis. In spite of this negative result it was felt that the situation among the galagos in Uganda called for further study, while in Kenya it obviously required investigation at the earliest date possible.

The first work undertaken was a survey of the mosquitoes, wild Primates and human population in forest areas of the Coast Province of Kenya. The work was carried out by Lumsden and the writer, the former working mainly on the mosquitoes and the latter mainly on the Primates. The first area studied was Gede, on the coast. The dry season had been selected, as it was important to find what sort of populations of adult mosquitoes persist at such times. It was found that, apart from the species breeding in mangrove (whose flight range extends inland for a short distance only) breeding was virtually at a standstill, and very few adults indeed could be found (Lumsden, 1955b). The new samples of monkeys and galagos, when combined with those previously obtained in the Gede area, gave a final immune rate of 3/57 (5 per cent) in the monkeys, and 12/61 (20 per cent) in the galagos. Eight mammals of other groups were included as a control. All were non-immune. In the samples of Africans studied many immunes were found, but in most cases it was not possible to exclude the possibility of vaccination having taken place. In one or two of the smaller children infection with the virus may have been responsible, but even here it was difficult to be certain. This matter has been discussed fully by Lumsden (1954) in a review of the results of vaccination campaigns in Kenya.

The work at Gede was followed by studies at Taveta, on the Kenya-

Tanganyika border. Taveta lies at the foot of Mt. Kilimanjaro, and here, in the middle of a wide area of dry bush country, is a very dense patch of high-canopy forest, which is maintained principally by hill-foot seepages. These appear in the form of flowing springs with a considerable output of clear, very cold water. Snow water from the high levels on Kilimanjaro is trapped under the sheets of volcanic rock and emerges only where these sheets end at Taveta. The local tribe, the Wataveta, live mainly in the dense forest around the springs, and have numerous banana plantations actually within its boundaries. A. simpsoni is so abundant in the forest and plantations as to be a pest. The biting-habits in this area have been discussed above. Monkeys are also very common.

Here once again, and for the same reason, the results of a human survey were difficult to appraise. The results in the monkey sample were, however, very clear-cut. Previous workers at Taveta had submitted monkey sera for test and, when this sample was combined with that obtained by the writer, the total number tested successfully reached 49 specimens. Not one was immune. On the other hand, though only 5 galagos (G. crassicaudatus panganiensis) were obtained during the visit to Taveta, one was found to be immune to yellow fever virus.

An interesting note from Taveta was that Lumsden found A. africanus in this tiny isolated forest patch - its presence so far east had not been suspected. As usual, biting by day was mainly at ground level. On a tree platform, however, the evening peak was not apparent. At this level also most of the specimens were taken by day. Lumsden (1955b) considers that these results indicate a difference in biting-habits and suggests that this may be the reason for the absence of immunity among monkeys. The writer feels that much more work would be required before such a conclusion could be accepted. In the first place, the sample was very small indeed (only 11 A. africanus from the tree-platform and 8 from ground level). Secondly, the platform used was not, in the writer's opinion, well placed for catches of A. africanus. Within his experience this mosquito bites mainly in the densest and leafiest parts of the canopy. This having been established, all the later Bwamba platforms (including all those used by

Lumsden) were sited with this point in mind. The same has proved true in all the numerous forest localities in Uganda which have now been studied and which are discussed in the foregoing section. The platform built by Lumsden for the present work at Njoro in the Taveta Forest, where all the A. africanus were obtained was, however, only 12.7 m. (about 42 feet) above ground level, whereas the canopy in the area concerned was very high indeed, probably at least 100 feet. Lumsden (loc.cit.) describes the platform as well situated among foliage. The writer's opinion is that it was placed in a fairly open part of the understorey, and not in a place where he personally would have expected to take many A. africanus, no matter how abundant this species might be at higher levels. The available evidence is not, therefore, considered adequate to establish that at Taveta the biting-habits of A. africanus are aberrant.

By the time that the work at Gede and Taveta had been completed, it had become evident that in Kenya an epidemiological picture was to be found which seemed to differ widely from that found further west. While, as in Uganda (outside Bwamba), human infections were apparently few and scattered, this also applied in the case of the monkeys, whereas in Uganda monkey infections are common and widespread. Thus there was no evidence to suggest that the infection in Kenya monkeys was other than sporadic, and there was no evidence whatever of enzootic monkey yellow fever. On the other hand, infections in galagos were not uncommon, and obviously it was necessary that these animals should be adequately sampled before an area was declared free of virus infection. Considering the total sample up till the time concerned, the following figures were obtained:

	Immunity Ratio	Per cent Immune
Monkeys	5/344	1.5
Galagos	14/103	13.6

All the galagos, however, came from broad areas known to be infected, whereas the monkey sample included specimens from areas where virus probably does not occur.

In the Coast Province, the only area where both groups are well represented in the sample, the figures were as follows:

	Immunity Ratio	Per cent Immune
Monkeys	3/113	2.7
Galagos	14/102	13.7

Finally, when the discussion is confined to areas where yellow fever in animals is known to occur, the results were:

	Immunity Ratio	Per cent Immune
Monkeys	5/271	1.8
Galagos	14/81	17.3

In this last example, the rate in galagos is almost ten times that in monkeys. These very striking results have been discussed more fully elsewhere (Haddow, 1952c).

It remains to add that further samples have been obtained from Kenya since that time. Work in the forest areas of the Central Province has been held up by the terrorist activities of the Mau Mau Society. One galago (G. crassicaudatus kikuyuensis) has, however, been obtained in that area. It was non-immune, as were two G. senegalensis braccatus from Kitui, another locality in the central highlands. A further small sample was obtained by the writer on the coast, consisting of 1 G. crassicaudatus lasiotis and 9 G. senegalensis zanzibaricus. One of the latter was immune to yellow fever virus, and this was a point of major interest as the animal concerned came from the almost waterless thornscrub wilderness between the Sabaki and Tana Rivers. In this area even waterholes are seasonal, and the ungulate fauna consists mainly of species such as the fringe-eared oryx (Oryx beisa annectens), the dik-dik (Rhynchotragus kirki nyikae) and the lesser kudu (Strepsiceros imberbis australis) which do not seem to require the constant presence of water (apart, of course, from that in the herbage, or occurring as dew). Here there could be no question of the indefinite persistence of an infection in mosquitoes and mammals as, for perhaps most of the year, mosquitoes are absent.

The total results for Kenya, as they stand at the time of writing, are as follows:

	Immunity Ratio	Per cent Immune
Monkeys	5/371	1.3
Galagos	15/113	13.3

The general conclusions drawn from the work in Kenya were thus that galagos appeared to be the main mammalian hosts of the virus in this area. As in some localities at least there seemed no possibility of the virus being maintained by mosquitoes the question again came to the fore as to whether there might not be another arthropod vector belonging to a different group. It was, in fact, concluded that there must almost certainly be some such vector. This view was borne out by the fact that infections in monkeys and man were, at the most, sporadic, and this became more readily explicable if it were postulated that infection in mosquitoes occurred only occasionally - almost, it might be said, fortuitously.

It may be mentioned that galagos must in any case be poor hosts for mosquitoes. During the day the various subspecies of G. crassicaudatus, the greater galago, sleep inside large roughly spherical nests formed from loose accumulations of leaves and twigs. The lesser galago, G. senegalensis, sleeps in tree-holes which may or may not contain a leafy nest. The smallest of the galagos, G. demidovii, which is essentially a rain-forest species, sleeps by day in leafy nests or, in some areas, under bark or in tree-holes. Thus, during the day, all three East African species are to a large extent protected from mosquito attack. By night they are intensely active. In the case of G. crassicaudatus and G. senegalensis the writer has shown that activity goes on throughout the night. This was done by confining specimens singly in a cage whose floor was suspended from rubber bands, even small movements causing it to rise and fall. The floor was connected by a wire to a pen writing on a recording drum with a 24-hour time scale. In addition to this direct experiment, the experience with large outdoor cages at Entebbe has shown that activity continues throughout the night. These cages are dimly lit to attract insect food, and as the galagos become fairly tame, observation is easy.

Thus by night galagos are much less suitable hosts for mosquitoes than are monkeys, which are asleep at this time. It is worth noting that their time of maximum activity - during which it is unlikely that they will be bitten - is the period just after sunset, the peak period for biting activity in A. africanus.

The results of the surveys in the Coast Province of Kenya drew attention to the fact that in the so-far almost unsampled Northern Province of Uganda there were extensive areas of dry almost semi-desert country. In particular Karamoja District seemed to call for investigation. This enormous district forms more than half of the border between Kenya and Uganda, and stretches northward to the Sudan. Parts of it are very dry, with bare rocky hills, succulent plants (euphorbias, aloes, Stapeliads, etc.) and in such areas hyraxes (Procavia habessinica meneliki and Dendrohyrax (Heterohyrax) brucei brucei) are the dominant mammals, living in large communities in the rocky cliffs and hills, and forming the main prey for specialised predators, such as Verreaux' eagle (Aquila verreauxi) and the fan-tailed raven (Rhinocorax rhipidurus). Other areas are mainly of the orchard bush type, with abundant plains game and game birds. The wide stretches of gently undulating country which make up most of the district are bounded along the Kenya border by the bare and jagged hills which rise from the lip of the main scarp of the Great Rift Valley, and similar ranges form the Sudan border. In addition, there are ten main isolated mountain masses rising from the plains. Most of these are volcanic, and several reach heights of over 10,000 feet. The inhabitants of the district, collectively known as Karamojong, are very primitive, usually naked, pastoral people. In this loose conglomeration of allied tribes cattle raiding and blood feuds are still of regular occurrence, and Karamoja is not yet open to the general public. The Karamojong make definite seasonal migrations, mainly in order to drive their huge herds of cattle, goats and sheep to the wetter and better pastured areas during the dry seasons.

During 1951 a first visit was paid to Karamoja. A sample of human sera from the area had previously been obtained, and this collection of 282 specimens included 9 positive sera. As two of these were known to have

come from ex-soldiers who had been vaccinated, the final result was 7/280, or 2.5 per cent. Though all the immune individuals were adults, it seemed at the least exceedingly unlikely that all had been vaccinated, as most Karamojong never leave the district in their lives, and no vaccination is carried on there (Ross, 1951). In this 1951 visit the entomological work was carried out mainly by Lumsden and the mammal work mainly by the writer. Lumsden took A. simpsoni biting in the area near Moroto and Lotome in central Karamoja, but did not find A. africanus in the forest round the base of Mt. Moroto. The writer took this species, however, in riverside thicket at Kaabong, in the extreme north and in the patchy forest round the base of the Labwor Hills in the extreme west of the district. He also obtained A. simpsoni at Kaabong, and A. luteocephalus, a very close relation of A. africanus, in forest at the base of Mt. Kadam in the extreme south of Karamoja. Obviously, however, mosquitoes could not maintain any indefinitely continued cycle of yellow fever in Karamoja as, apart from the seasonal scarcity of mosquitoes, the monkey population is sparse. Bands of grey monkeys (C. aethiops arenarius) occur in the riverside thickets and in the small belts of "dry forest" round the main mountain bases. Patas monkeys (Erythrocebus patas pyrrhonotus) range through the opener bush, but are nowhere very abundant. Baboons (Papio doguera ssp.indet.) are fairly common, but confined mainly to the areas with rocky hills.
In the extreme south there is a narrow belt of dense swamp-forest at the base of Mt. Kadam, and here the Elgon colobus (Colobus abyssinicus matschiei) and Brazza monkey (Cercopithecus neglectus) occur. Generally speaking, however, monkeys are sparsely distributed in Karamoja, at least when compared with the rest of Uganda.

During the first work in Karamoja over 30 monkeys were obtained, and others were collected during a subsequent visit. The sample stands at present at 36 monkeys, not one of which has shown immunity to yellow fever virus, though the group includes 22 adult and old specimens. The distribution of the sample is good, as monkeys have been obtained at Loyoro in the north, at Moroto and Lotome in central Karamoja, and at the base of Mt. Kadam in the extreme south. "Dry" mountain forest, riverain gallery forest, thorn scrub and dense, humid swamp-forest have been included in the habitats sampled. Thus, in future work, it is not felt that more than an occasional monkey (if any) is likely to show immunity.

In the case of the galagos the results were strikingly different. It took a good deal of time to learn what was the most suitable environment in which to look for them, and what were the favourite species of trees, the type of tree-hole preferred, and so on. The only galago known to occur in Karamoja is the small open country species G. senegalensis albipes, and though it is hardy in captivity when established, a good deal of care is necessary in the first few days or many will die. This was not at first realised. In this first collection, therefore, the sample obtained was rather small, and some of the animals died before they could be tested. Finally, however, successful tests were made on 23 specimens, and among these were 7 immune sera - a rate of 30 per cent positive. It was now obvious that in Karamoja a situation existed which was entirely different from that found in most of Uganda, but extremely similar to that found in the adequately sampled areas of Kenya. It should be mentioned that yellow fever infections in G. senegalensis have been studied in West Africa (see Bugher, in Strode, 1951). The animal reacts in much the same way as an African monkey - it circulates virus for a day or two and subsequently becomes immune.

During this first experience in Karamoja Lumsden worked on nest material from tree-holes in which G. senegalensis had been found, and this proved to be heavily infested with mites. Many of these were subsequently recognised as saprophytic, but a blood-sucking mite, later described as Liponyssus galagus was also regularly present. Lumsden also obtained some sand-flies (Phlebotomus spp.) from tree-holes. The question now arose as to whether nest parasites might be the vectors of the infection among galagos in the drier areas, and it was decided that more work in the field was required, particularly with reference to this aspect of the problem.

In survey work generally it is usual to include a few mammals of non-primate groups as a control. Protection tests, formerly carried out by senior staff are now generally done by a technician and, as a rule, the person doing the work is not told which sera are which till the test has been completed. In the first sample from Karamoja two monitor lizards (Varanus sp.), two Sudanese hedgehogs (Atelerix pruneri hindei) and fifteen hyraxes, belonging to the two species mentioned above, were included. None showed antibodies to yellow fever. The hedgehogs were included as this species is known to be susceptible under laboratory conditions (Findlay, Hewer and Clarke, 1935; Dick, 1952b). The hyrax is also considered as of a certain interest as, after the Nuba Mountains epidemic, an immune specimen was found among a small sample sent from that area for test at Entebbe, and it was subsequently shown that the hyrax, when experimentally infected, circulates a small quantity of virus and becomes immune (Smithburn and Haddow, 1949b). Lumsden and Buxton (1951) had previously obtained six hyrax sera in the West Nile District, and these also were non-immune. It may also be mentioned that a further nine hyrax sera from Karamoja have now been tested, once more all specimens being negative. The sample of hyraxes from northern Uganda now stands, therefore, at 30 animals - all non-immune - and it seems unlikely that hyraxes can be of importance in the epidemiology.

Since the time of the first Karamoja survey the time available to the writer for field work at any distance from the Institute has been very limited. He has therefore concentrated on this district and on other limited areas in Kenya and Uganda, while Lumsden has undertaken the work in other countries. The second Karamoja survey was undertaken to study the situation among the galagos in further detail. No other animals were collected, and mosquito work was kept to a minimum. The main object was to obtain detailed information about the animals themselves, and extensive records were kept of weights, measurements, tooth replacement and wear, etc. It was considered particularly important to find whether the infection seemed likely to be transmitted in the nest. In this event it was anticipated that individual family groups would show either a high incidence of immunity regardless of age, or none at all. The biological data were collected by the writer. A colleague bled the animals, using a new technique. Unfortunately this method proved unsatisfactory and a large number of the specimens became contaminated and toxic to mice, the test results being unreliable in consequence. Of 120 G. senegalensis bled only 63 could be tested successfully. Three of these were immune to yellow fever. One came from Moroto and one from Lotome in central Karamoja. The third, however, came from Kaabong in the extreme north, this being the most northerly positive test so far obtained in Uganda galagos. Though the large number of untestable specimens eliminated any chance of studying the distribution of immunity by family groups, a great deal of other information was obtained. Examination of the contents of the alimentary canal in 100 specimens revealed that G. senegalensis is almost exclusively insectivorous. Vegetable debris was found only in about 10 per cent of specimens. The commonest recognisable insect fragments were from Lamellicorn beetles and from caterpillars. Other commonly eaten food included ants, spiders, Asilid flies, antlions and termites. In a few cases scorpion remains were found. The conclusion was reached that G. senegalensis is not so exclusively arboreal as had been thought, and that at least some feeding takes place on the ground. Family parties consisted of groups ranging from two to five. Solitary animals were common. A good collection of embryos of varying sizes was obtained, and it was noted that these all occurred singly, whereas most of the textbooks on African animals state that the young are born in pairs (see for example Rode, 1937).

Some very interesting results were obtained with reference to the incidence of ectoparasites. Roughly half the sample was obtained in central Karamoja. Very few ectoparasites were obtained on the animals from this area, probably because the method of search was inadequate. The second half of the sample was collected at Kaabong in the far north, and here the animals, after being anaesthetised and bled out, were weighed, measured, etc., and then brushed carefully above a large white enamel tray before being skinned. This method at once revealed that a very high proportion are infested by the blood-sucking mite Liponyssus galagus and by the louse Lemurphthirus galagus. Thus of 54 carefully searched 35 (65 per cent) carried mites and 39 (72 per cent) carried lice. This observation was in striking contrast to those made on monkeys, where it had been found that among wild specimens ectoparasites are very scarce indeed. Thus there now seemed to be a strong enough prima facie case for detailed study of the galago ectoparasites as possible vectors of the virus.

There was no further work in Karamoja till 1955, when M. C. Williams collected a sample of galagos in the south-west of the district. From his sample came 11 valid tests, with 1 immune specimen. The writer then began work once more in the north, collecting galagos, while two colleagues collected other animals, birds (for work on other viruses) and cattle sera (for test against Rift Valley fever virus). The galago collections covered the country along a 50-mile stretch of road, and 45 were obtained, 12 being immune to yellow fever virus. All the immune specimens came from a section of the sample strip only about 8 miles long.

When the sample was studied with reference to family grouping, the following results were obtained: There were 32 non-immune animals, of which 13 had been found as single specimens in tree-holes, there were three family groups of two, three groups of three, and one group of four. The immune specimens all occurred within five groups, as follows:

1. A single animal, immune.
2. A group of two, both immune.
3. A group of three, all immune.
4. A group of three, two immune (the non-immune specimen was very young, probably under one year old).
5. A group of four, all immune.

Thus of a total of 13 animals in these groups twelve showed immunity. It therefore appeared that if one animal of a group acquired the infection, it was likely that the other members would also acquire it. This pattern is, as mentioned above, very characteristic of infections carried by nest parasites and is not what one would expect were mosquitoes responsible for the transmission. Further, this and previous experience in Karamoja had shown that the distribution of immune specimens is very local, and this also suggested a flightless vector or at least a vector which does not fly far.

The writer's work in Karamoja has not yet been written up in detail, only preliminary notes in the annual reports of the Institute having so far appeared (Haddow, 1952d, 1953; Haddow, Williams and Mims, 1955).

During the same period surveys carried out by Lumsden in other parts of Africa revealed that infections in galagos are to be found in very widely separated areas. In dry bush country in southern Tanganyika he obtained one immune G. crassicaudatus in a sample of five. Thirteen G. senegalensis and G. demidovii from the same area were all negative, as were seven monkeys belonging to three species (Lumsden and Hewitt, 1954). Another East African survey was carried out on the islands of Zanzibar and Pemba, following the discovery of two immune specimens in a sample of human sera sent to the Institute by the local authorities. Here Lumsden obtained a very large sample of G. crassicaudatus agisymbanus, the result showing 9 immune specimens among 253 tested. There were no immunes among 27 G. senegalensis zanzibaricus from the same survey, and only one among 51 monkeys. There were no immunes among 27 other mammals tested. These figures are quoted from the table given by Lumsden, Ellice and Hewitt (1955). Finally Lumsden made a survey in Northern Rhodesia and Nyasaland. As mentioned above, South African workers had collected a large monkey sample in Northern Rhodesia and all had been negative. The results of tests on Lumsden's collections are not all available as yet, but the completed figures are as follows:

	Immune Ratio	Per cent Immune
G. crassicaudatus	7/28	25%
G. senegalensis	24/185	13%
Monkeys	0/5	-
Other mammals	0/24	-

These figures (Lumsden, Ellice, Mason and Hewitt, 1956) are in agreement with those obtained in the dry areas of East Africa discussed above, the overall rate in the galagos (31/213, or 15 per cent) being closely comparable to that obtained in Karamoja (23/112, or 21 per cent) and in Kenya (15/113, or 13 per cent).

In the Southern Sudan various workers, notably Mahaffy, Smithburn and Hughes (1946) had shown that there were various places with a high incidence of immunity in man and in view of this fact surveys in animals have been undertaken by Kirk and Haseeb (1953) and by Taylor, Haseeb and Work (1955). Here, most interestingly, the picture was not, as had been anticipated, similar to that in Karamoja, with a relatively high incidence of immunity in galagos and little or none in monkeys. Instead, a situation closer to that found in the wetter areas of Uganda was encountered. Kirk and Haseeb found two immune monkeys in a sample of 24 and no immune galagos in a sample of ten (G. senegalensis). Taylor, Haseeb and Work found only one immune galago in a sample of 52, whereas in their monkey sample the rate was 40/101, or approximately 40 per cent immune (they list various inconclusive tests, which are not included among the figures quoted here). Their results from El Muglad and Gogrial, which were as follows, were of particular interest:

El Muglad	Gogrial
Monkeys 11/17	Monkeys 28/31
Galagos 0/9	Galagos 1/17

The incidence of immunity among monkeys at Gogrial is, therefore, one of the highest ever recorded. The overall rate found by these workers in grey monkeys (Cercopithecus aethiops ssp. indet.) was 77 per cent, and in baboons, all of which came from Gogrial, the rate was 94 per cent.

It may be mentioned that among early surveys that of Findlay and MacCallum (1937) had included three G. senegalensis from the Nuba Mountains, all non-immune. When, therefore, the results of all the animal surveys in the Sudan are combined (valid tests only being included) there has only been one immune G. senegalensis in a sample of 65.

It is interesting to note that on the other side of Africa Bugher (in Strode, 1951) found no positives among 50 G. senegalensis from Zaria in the Nigerian highlands. Unfortunately the situation among the monkeys of the same district - if indeed there be any in that particular area - is not known.

It is considered that there is now abundant evidence that G. senegalensis and G. crassicaudatus are important mammalian hosts of yellow fever virus in many parts of Africa. The mechanism of transmission among them remains, however, obscure. After overcoming considerable initial technical difficulties, Lumsden attempted to infect the bloodsucking mites common on bush babies. He was able to demonstrate virus in them immediately after the infective meal, and up to four days thereafter, but beyond this time limit he has not been able to show persistence of the virus and, up to the time of writing, no transmission to animals has been achieved (Lumsden, Hewitt, Ellice, Mason and Santos, 1955; Lumsden and Ellice, 1956). More work is required before a final decision is reached, but it now appears unlikely that this line of investigation, which initially appeared so promising, will provide the solution to the problem. Other lines await study. Present evidence suggests infection in the nest and in this connection it must be remembered that lice abound on galagos. These are probably the next parasites which will be studied, but this is not considered a particularly hopeful line, as lice have not so far been incriminated as vectors of any virus infection. They do, of course, transmit the rather closely allied rickettsiae of epidemic typhus, but these organisms are much larger and in fact differ in many ways from the viruses, to which it may be added that transmission is not directly by bite.

Another line calling for investigation is the possibility that Phlebotomus spp. may be the vectors among galagos. They do occur fairly freely in tree-holes, possibly in greater numbers than suspected, as these tiny insects are notoriously difficult to find. The fact that yellow fever virus has actually been isolated from Phlebotomus spp. (Smithburn, Haddow and Lumsden, 1949b) is not to be forgotten. A further matter of importance is that they show considerable powers of survival in hot dry country. In the Sudan they may be found in cracks in apparently dry ground, and much the same applies to the Middle East and to Kenya, where they have been found in animal burrows and the tunnels in termite mounds during very hot dry weather (R. B. Heisch, personal communication). Further, it must not be forgotten that sandflies have already shown the capacity to transmit virus transovarially from generation to generation in the case of sandfly fever, as has been mentioned above. At the moment, therefore, the study of sandflies seems the most hopeful line to follow. Work is, however, likely to proceed slowly as there are great technical difficulties in carrying out transmission experiments with these insects, and even in maintaining sandfly colonies in the laboratory.

AN APPRAISAL OF THE PRESENT SITUATION AND NOTES ON TRENDS OF INVESTIGATION

It is only since the dry country work discussed above was begun that the complexity of the African yellow fever situation has become fully evident, the observed differences between the different areas being very great.

Thus, in the great rain-forest of the Congo Basin, which extends into the west of Uganda as the Semliki Forest, it is probable that the epidemiology is everywhere similar to that found in Bwamba (as summarised above on pp. 140-141). A good deal is known of the yellow fever of this enormous forested area, which is probably the true home of the virus, the studies in Uganda having been directed mainly toward the mosquito and mammalian hosts, while those in the Congo have concentrated mainly on human infections, studied principally by means of the viscerotomy service and by protection test surveys (see, for example, Liégeois, 1944, and Liégeois, Rousseau and Courtois, 1948). It has become evident that in these great forests the human infection is usually mild, only occasionally fatal, and seldom recognisable clinically as yellow fever. The people, in other words, may be showing evidence of adaptation to the virus over a very extended period of time. Except round such areas as the mouth of the Congo where European intervention and seaport conditions have materially altered the environment, there seems to be no history of epidemics with that rapid spread from man to man which seems to be associated with the generalised production of the violent "yellow jack" type of infection.

North and west of the Congo Basin, in the area round the Gulf of Guinea, a different picture is found. Here there is less forest, but the forested areas are still very extensive. In countries such as Nigeria and the Gold Coast monkey-to-monkey infection is known to occur. Here, however, human epidemics are still common and dangerous, with a high mortality rate (for a summary of this situation see Macnamara, 1955) and this aspect of the problem has naturally dominated the studies of the virus in West Africa. It is of course to be remembered that West African villages may have thousands of inhabitants, very closely concentrated, and purely African cities may be very large indeed (Kano has over 50,000 inhabitants). Thus there are opportunities for rapid man-to-man spread of the infection which do not occur elsewhere in the equatorial belt in Africa. In the Belgian Congo, it is true, there is a good deal of regimentation of the people into villages, for administrative reasons, but these units are small, consisting of hundreds, not thousands, of people. In most of East Africa the population is still more dispersed, and the family hut-group or individual hut is still overwhelmingly the commonest unit, except in the vicinity of the larger towns. The other great difference between West Africa and the rest of the area under discussion is that in West Africa A. aegypti seems everywhere to be a domestic anthropophilic mosquito, as has been shown by the work of numerous investigators.

In eastern Africa, after the forest belt has been left, several pictures are presented. First there is that found in many of the wetter areas of Uganda, where there is widespread infection among the monkeys, but only occasional infection in man. The scarcity of human infections is unquestionably due to the fact that neither A. simpsoni nor A. aegypti bites man freely in most of the area concerned. In the drier country, there is a still lower incidence of infection in man (often there is no sign of human infection at all) and little or no infection among the monkeys. Here, though mosquitoes carried long distances by wind and convection might be responsible for periodic reintroduction of the virus - perhaps at long intervals - they could not be responsible for its indefinite maintenance. In such country the bush-babies (Galago spp.) are the only mammals which seem to be involved to any great extent in the epidemiology. The limited evidence at present available suggests that infection occurs in the nest. The vector is unknown. Mites, lice and sandflies are suspect, and investigations on these have been begun. This particular epidemiological picture has been found both in the dry areas of Uganda, in Kenya, and in Tanganyika. It is anticipated that further work may show it to be a very widespread epidemiological type.

From the foregoing it is obvious that in areas where there is clear evidence of yellow fever among the galagos the monkeys are not, or are only slightly, involved. Examples of this situation are to be found in

Karamoja, the Kenya Coast and Northern Rhodesia. Other areas considered likely to show a similar picture still lack in some cases an adequate monkey sample and in others an adequate sample of galagos, to settle the matter. In most of the areas where the galago appears to be the main mammalian host there is little evidence of yellow fever in man. Here, however, there are exceptions, typified by Northern Rhodesia and Nyasaland, where the only wild mammalian hosts so far found are the galagos, and where monkeys appear to play no part, but where human infections seem to be relatively common.

Similarly, in monkeys, a high rate of infection is usually characteristic of the wetter areas. Here also, however, a striking exception is to be found, there being an exceedingly high immune rate among the monkeys of the rather dry country of the southern Sudan, where man is also heavily involved. Galagos are apparently of little importance in this area. It will not be forgotten that the greatest yellow fever epidemic ever recorded in Africa occurred only a few years ago in this country.

This complicated and often apparently contradictory picture outlines the present very inadequate state of knowledge concerning the epidemiology of yellow fever in Africa. It remains to enquire what, if any, factor is common to all the situations just summarised. From the point of view of the mammalian hosts it is pointed out that the galagos occur in all the areas involved. The large galago, G. crassicaudatus is essentially a creature of the forest fringes and denser woodland while G. senegalensis is an inhabitant of the more open "orchard bush" type of country. The third common species, Galagoides demidovii, is essentially an animal of the rain-forest, though it is also prevalent in the denser types of second-growth. Recently Lumsden has found it in dense bush on the Tanganyika coast, and this seems to indicate a much wider range - both in geographical distribution and in habitat - than had been suspected. This tiny animal is shy, inobtrusive, and difficult to capture. In its arboreal habitat it is rarely seen, more particularly as it is essentially, even if not exclusively, nocturnal. Very little is known of G. demidovii, but it is anticipated that further work will almost certainly show it to be common throughout the main forest areas, and probably far beyond their borders. Between them, the various species of galago cover the entire yellow fever area of Africa, and sometimes all three common species may be found in a single relatively small stretch of country. The rarer or more localised species such as Galago alleni and Euoticus elegantulus have not yet been studied in relation to yellow fever, and unfortunately do not occur within the area accessible to the Entebbe Institute staff.

G. demidovii has not so far received much attention, mainly because Bugher (in Strode, 1951) has reported anomalous results in a long series of experimental laboratory infections. Bugher's results differ from those obtained with every other primate so far tested, whether from Africa, America or the East, and he himself suggests that confirmatory work is required. In his sample many specimens did not circulate virus, and some of those which did circulate did not subsequently show antibody. Bugher's results are available only in the shortest summary form and unfortunately it seems unlikely that the detailed results will ever be published, as he is now working in a different field.

An interesting clue to this otherwise puzzling situation has recently come from work carried out at Entebbe. Studies on captive galagos have shown that the antibody levels may fluctuate considerably and that a galago, some time after an experimental infection, may experience such a fall in antibody titre as to give an "inconclusive" result on test. The antibody may subsequently show a rise once more. This observation has been made by various workers at the Entebbe Institute, namely, G. W. A. Dick, R. W. Ross, W. H. R. Lumsden and the writer. The matter is now being thoroughly investigated by Dr. Max Theiler's team at the Rockefeller Foundation Laboratories in New York, using material sent from Entebbe. It seems, therefore, quite possible that immunity in a galago could fall below the level detectable by the test, the animal, none the less, being solidly protected against the virus. Such a situation probably occurs in other virus diseases also. Thus some (perhaps the majority) of Bugher's apparently non-immune yet refractory animals might have experienced an attack of yellow fever in nature with subsequent decline of antibody to a level where it was not shown by the protection test. Such animals would not, of course, circulate virus again. It may be mentioned, incidentally, that the intracerebral test employed in this work (Bugher, 1940) is not the most sensitive where low antibody levels are concerned.

A further clue came from an experiment carried out in 1952, when two G. demidovii thomasi were caught near Entebbe. Previous experience in Bwamba had shown that these animals are difficult to acclimatise and of those caught by the writer none had survived long enough to undergo a circulation experiment with yellow fever virus. He therefore requested R. W. Ross to bleed these specimens at once for protection test and thereafter to proceed at once with a circulation experiment without waiting to see the results of the test. Unfortunately, in the pre-experimental protection test both animals gave an inconclusive result. Such a finding may of course indicate a past attack of yellow fever with a subsequent fall of antibody. Equally, it may mean nothing. There was not enough serum left for a titration of antibody or even for a repeat test (these galagos are smaller than most squirrels and it is very difficult to obtain serum specimens of useful volume from them). The experiment, however, gave a most interesting result (Ross, 1953). The first galago circulated no virus, but by the tenth day after inoculation its antibody had risen from the "inconclusive" level to a definite positive, and between the tenth and seventeenth days a further very striking rise occurred. It is a legitimate deduction that this animal had experienced a yellow fever infection in nature, and that it had thus been apparently refractory where virus circulation was concerned. The rise in antibody indicated that the experimental inoculation of virus had acted as a "booster" dose. Such an antibody rise in man is, of course, the basis of much prophylactic vaccination against bacterial and virus diseases, booster doses being given to raise or maintain the antibody level. The second galago was still more interesting. It behaved in exactly the same manner as other species of the genus and as the African monkeys. It circulated virus for five days and subsequently became solidly immune. On two of the days when virus was circulated the titre was very high, 4.7 and 4.5 log LD_{50} per ml. serum (that is to say that on these days the amount of virus present in the serum was such that 50 per cent of inoculated mice would have been killed by the virus even when the serum was diluted approximately 1:50,000 and 1:30,000 respectively). This result showed clearly that, in spite of Bugher's findings, G. demidovii could well be involved in the forest cycle, as in some cases at least it can circulate virus in quantities adequate to infect even a very small arthropod vector.

It will be remembered that in appraising the role of monkeys as mammalian hosts of the virus in Uganda, it had been concluded that there might very well be some unknown cycle of infection and that monkeys might not be the basic hosts. It was felt that "the monkey-to-monkey cycle may possibly be, like the man-to-man cycle, the endpoint of some chain of incidents of which we know nothing" (Haddow, Dick, Lumsden and Smithburn, 1951). Were it to be established that G. demidovii were involved, then the basic animal host might at last have been discovered, as this tiny galago is probably present in very much larger numbers than the monkeys throughout the rain-forest areas. It is the mammal which most urgently requires further investigation as, in the writer's opinion, the sandflies, with their known potentiality to transmit at least one virus from generation to generation, are the arthropods most definitely calling for study. This view is, of course, based partly on conjecture, but such clues as are at present available, slender though they may be, do point to G. demidovii and the Phlebotomus spp. as the most hopeful potential hosts to investigate. The work will not be easy as both are technically difficult to observe, to collect in the field and to maintain in the laboratory. It is also necessary to remember how many seemingly hopeful lines of yellow fever investigation have come to nothing in the past. Should the projected studies fail, it will simply be necessary to begin once more on some other basis.

In conclusion it seems essential to set forth some explanation of the underlying reasons for the continuation of investigative work on yellow fever. The days when the virus was the agent of one of the most deadly epidemic diseases of the tropics and subtropics are probably at an end.

It is, indeed, difficult to remember that within living memory a general exodus from towns round the Gulf of Mexico and in the southern United States might follow the report of an approaching epidemic (and such epidemics occurred almost annually), that ports might be closed for months at a time and that at one stage yellow fever caused abandonment of all work on the Panama Canal. With the advent of an effective and fairly inexpensive vaccine it is now mainly a matter of personnel, finance, and public health organisation to halt an outbreak (though it is always to be remembered that the introduction of virulent virus to the crowded populations of the Far East might well begin one of the greatest epidemics in recent history). This particular aspect, however, is one which, should the situation ever occur, will be dominated by political and financial considerations and by vaccine supplies. Such matters are entirely beyond the scope of this communication, and it must be admitted that, in Africa at least, the main importance of continued study of this virus is not directly related to the occurrence of human infection. Yellow fever has become the model for the study of arthropod-borne virus infections. This aspect has been stressed by Kerr (1953) and it is a great deal more important than may at first sight appear. Much more is known about yellow fever than about any of the other arthropod-borne virus infections, and most workers in this field feel that, could we but elucidate the whole epidemiology, much light might be cast on similar less well studied virus infections.

In the case of the bacterial diseases the trend of modern work has usually resulted in a simplification of the picture. It is true that in many cases sub-grades, local strains, virulent and avirulent types, etc., have been recognised. Generally speaking, however, once the agent has been isolated, the trend has been toward the investigation of detail, the study of local conditions and host populations, and the exploration of the social and economic fields to find the most effective combat methods permissible within some given economy. Much the same has been true of the virus diseases spread from man to man by droplet infection or by contagion. In the arthropod-borne virus infections, however, the trend has been entirely different. Here, in the case of almost every adequately studied infection the picture - apparently fairly simple at first - has become increasingly complex and obscure as knowledge has accumulated. Almost all of the many viruses concerned may cause epidemics in man or in animals from time to time and during such periods may be isolated fairly easily from the mammalian hosts and the arthropod vectors. In the inter-epidemic periods, however, they virtually disappear and at such times only the most assiduous work - with very adequate financial backing - will lead to isolations in the field. The persistence of virus through such periods is one of the greatest problems facing the investigator, and it is one of very great importance from the practical standpoint of public health, particularly in the tropics.

As stated above, yellow fever is by far the most thoroughly studied of all these infections, the reason being, of course, its overwhelming importance up till a few years ago. Time and again the epidemiology has been worked out, and the whole problem "solved". It was solved in Central America and the Caribbean in the early years of the century by the discovery that the virus was transmitted by A. aegypti, and that the same epidemiological picture was to be found in West Africa. It then seemed that eradication of the disease from the world might be merely a question of finance and health administration, with the total destruction of A. aegypti as the object. After this had been achieved, at enormous cost, over very great areas of the New World, the discoveryof "jungle yellow fever" reopened the entire question, and shortly afterwards the recognition of animal-to-animal forest cycles in Africa showed that there also the problem was much more complex than had been realised. The writer will not readily forget that on the day on which the identification of the yellow fever virus obtained from A. africanus was completed, a colleague wrote that the epidemiology of the rural and forest cycles of yellow fever in Africa was now (to quote his actual words) "in the bag". Subsequent studies have shown how far this was from the truth. It is a sad admission, but it must be made, that while what might be called the superficial epidemiology of this infection has now been worked out in fair detail over a large part of Africa, there are the strongest reasons to believe that underlying and perhaps very important factors await discovery. This is still, of course, true of all the known arthropod-borne virus infections. Investigating these - and perhaps more particularly yellow fever - the worker feels that he is advancing slowly and with many false casts into a labyrinth of increasing complexity. So far, the study of arthropod and animal behaviour has, almost without exception, provided the clues by whose help advance has been made possible. Only by continued study of the natural history of the hosts and vectors, both in the field and in the laboratory, can that basic information be gained which must precede any final explanation of the epidemiology of yellow fever in Africa.

ANNOTATED LIST OF PAPERS SUBMITTED

In submitting the following papers, the writer wishes to mention that he bases his application mainly on Nos. 1, 2, 3, 6, 7, 8, 10, 11, 12, 13, 15, 17, 18, 21, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 38, 39, 40 and 41. In the work described in the others he played a smaller part. They are included as supporting papers. The extent of the writer's contribution in cases of collaboration is indicated in the list below.

The text also contains references to recent work which so far has been published only as short notes in annual reports of this Institute. These reports are not submitted as the work is in most cases incomplete and will eventually be presented in fuller form for publication elsewhere.

(1) HADDOW, A. J., 1942a. "The mosquito fauna and climate of native huts at Kisumu, Kenya". Bull. ent. Res., 33, 91-142.

(2) HADDOW, A. J., 1942b. "A note on the predatory larva of the mosquito Culex (Lutzia) tigripes Grandpré and Charmoy (Diptera)". Proc. R. ent. Soc. Lond. (A), 17, 73-74.

(3) HADDOW, A. J., 1943. "Measurements of temperature and light in artificial pools, with reference to the larval habitat of Anopheles (Myzomyia) gambiae Giles and A. (M.) funestus Giles". Bull. ent. Res., 34, 89-93.

(4) SMITHBURN, K. C. & HADDOW, A. J., 1944. "Semliki Forest virus. I. Isolation and pathogenic properties". J. Immunol., 49, 141-157. (A.J.H. played a small part - the collection of the mosquitoes and animals. All the inoculations and laboratory studies were carried out by K.C.S.).

(5) SMITHBURN, K. C., MAHAFFY, A. F. & HADDOW, A. J., 1944. "Semliki Forest virus. II. Immunological studies with specific anti-viral sera and sera from humans and wild animals". J. Immunol., 49, 159-173.
(K.C.S. again played the main part. A.F.M. and A.J.H. collected the Bwamba sample of human bloods and A.J.H. collected almost all the wild animals).

(6) HADDOW, A. J., 1945a. "The mosquitoes of Bwamba County, Uganda. I. Description of Bwamba, with special reference to mosquito ecology." Proc. zool. Soc. Lond., 115, 1-13.

(7) HADDOW, A. J., 1945b. "The mosquitoes of Bwamba County, Uganda. II. Biting activity with special reference to the influence of microclimate". Bull. ent. Res., 36, 33-73.

(8) HADDOW, A. J., 1945c. "The mosquitoes of Bwamba County, Uganda. III. The vertical distribution of mosquitoes in a banana plantation and the biting cycle of Aëdes (Stegomyia) simpsoni Theobald". Bull. ent. Res., 36, 297-304.

(9) SMITHBURN, K. C., HADDOW, A. J. & MAHAFFY, A. F., 1946. "A neurotropic virus isolated from Aëdes mosquitoes caught in the Semliki Forest". Amer. J. trop. Med., 26, 189-208.
(K.C.S. carried out the laboratory work. A.J.H. carried out the

field work on mosquitoes and monkeys and was responsible for the grouping of sera by area. A.F.M. and A.J.H. collected the human sera).

(10) SMITHBURN, K. C. & HADDOW, A. J., 1946. "Isolation of yellow fever virus from African mosquitoes". Amer. J. trop. Med., 26, 261-271.
(K.C.S. carried out the laboratory work, A.J.H. the field work).

(11) HADDOW, A. J., 1946. "The mosquitoes of Bwamba County, Uganda. IV. Studies on the genus Eretmapodites Theobald". Bull. ent. Res., 37, 57-82.

(12) HADDOW, A. J., GILLETT, J. D. & HIGHTON, R. B., 1947. "The mosquitoes of Bwamba County, Uganda. V. The vertical distribution and biting-cycle of mosquitoes in rain-forest, with further observations on microclimate". Bull. ent. Res., 37, 301-330.
(A.J.H. initiated the work. J.D.G. and R.B.H. played a full part in building the platforms and in the 1944 catches. A.J.H. carried out all the 1945 catches and all the climate work. A.J.H. and J.D.G. wrote the paper).

(13) HADDOW, A. J., SMITHBURN, K. C., MAHAFFY, A. F. & BUGHER, J. C., 1947. "Monkeys in relation to yellow fever in Bwamba County, Uganda". Trans. R. Soc. trop. Med. Hyg., 40, 677-700.
(A.J.H. collected most of the material, made the maps, wrote the paper and is responsible for most of the conclusions. K.C.S. carried out the protection tests. A.F.M. contributed some monkey sera. J.C.B. advised on statistics and worked out the equation on p. 696).

(14) SMITHBURN, K. C., HADDOW, A. J. & GILLETT, J. D., 1948. "Rift Valley fever. Isolation of the virus from wild mosquitoes". Brit. J. exp. Path., 29, 107-121.
(K.C.S. carried out the laboratory work. A.J.H. carried out the mosquito catches. J.D.G. took part in the last catch from which virus was isolated).

(15) HADDOW, A. J., 1948. "The mosquitoes of Bwamba County, Uganda. VI. Mosquito breeding in plant axils". Bull. ent. Res., 39, 185-212.

(16) DICK, G. W. A., BEST, A. M., HADDOW, A. J., & SMITHBURN, K. C., 1948. "Mengo encephalomyelitis. A hitherto unknown virus affecting man". Lancet, 255, 286-289.
(A.J.H. made the direct inoculations of mice and monkeys with serum from the human case).

(17) HADDOW, A. J., SMITHBURN, K. C., DICK, G. W. A., KITCHEN, S. F. & LUMSDEN, W. H. R., 1948. "Implication of the mosquito Aëdes (Stegomyia) africanus Theobald in the forest cycle of yellow fever in Uganda." Ann. trop. Med. Parasit., 42, 218-223.
(A.J.H. carried out almost all the field work. K.C.S. made the histological examinations. The others are included as they (particularly G.W.A.D.) had taken part in previous attempts to isolate yellow fever virus from forest mosquitoes).

(18) HADDOW, A. J. & DICK, G. W. A., 1948. "Catches of biting Diptera in Uganda with anaesthetised monkeys as bait". Ann. trop. Med. Parasit., 42, 271-277.
(A.J.H. initiated the work, made the catches and wrote the paper. G.W.A.D. worked out the method of anaesthetising the monkeys, and was present during the catches to administer the anaesthetic as required).

(19) DICK, G. W. A., SMITHBURN, K. C. & HADDOW, A. J., 1948. "Mengo encephalomyelitis virus: isolation and immunological properties". Brit. J. exp. Path., 29, 547-558. (A.J.H. played a small part - the collection and identification of the mosquitoes from which isolations were made).

(20) SMITHBURN, K. C., HADDOW, A. J. & LUMSDEN, W. H. R., 1949. "Rift Valley fever: transmission of the virus by mosquitoes". Brit. J. exper. Path., 30, 35-47. (A.J.H. and W.H.R.L. made the mosquito collections and bred out males for identification. All the other work was carried out by K.C.S., as he was already immune to the virus, following an infection some years before).

(21) SMITHBURN, K. C., HADDOW, A. J. & LUMSDEN, W. H. R., 1949. "An outbreak of sylvan yellow fever in Uganda with Aëdes (Stegomyia) africanus Theobald as principal vector and insect host of the virus". Ann. trop. Med. Parasit., 43, 74-89. (K.C.S. carried out all the work at Entebbe. He and A.J.H. had collaborated equally over a number of years on this work and he here appears as senior author as A.J.H. was senior author of paper No. 17, on the same subject. A.J.H. was in charge of the sentinel programme and of most of the mosquito catching till early July. He made the inoculations of material from the first sentinels to come down, and also took charge of the catching and inoculation of most of the mosquitoes, up till early July. W.H.R.L. made trap catches during the earlier work and superintended the feeding of A. africanus on monkeys. He made all the catches and inoculations of Phlebotomus spp. From early July till October the entire field programme in Bwamba was in his hands).

(22) SMITHBURN, K. C. & HADDOW, A. J., 1949a. "The susceptibility of African wild animals to yellow fever. I. Monkeys". Amer. J. trop. Med., 29, 389-408. (A.J.H. played a very small part here - merely the collection and identification of the monkeys).

(23) SMITHBURN, K. C. & HADDOW, A. J., 1949b. "The susceptibility of African wild animals to yellow fever. II. Rodents, bush pig, hyrax and leopard". Amer. J. trop. Med., 29, 409-414. (Once again A.J.H.'s contribution was merely the collection and identification of the animals).

(24) SMITHBURN, K. C., MAHAFFY, A. F., HADDOW, A. J., KITCHEN, S. F. & SMITH, J. F., 1949. "Rift Valley fever: accidental infections among laboratory workers". J. Immunol., 62, 213-227. (A.F.M. and A.J.H. carried out the investigation of the first case here reported).

(25) HADDOW, A. J. & MAHAFFY, A. F., 1949. "The mosquitoes of Bwamba County, Uganda. VII. Intensive catching on tree-platforms, with further observations on Aëdes (Stegomyia) africanus, Theobald." Bull. ent. Res., 40, 169-178. (A.J.H. initiated the work and carried out the catches with A.F.M.'s help. A.F.M. did most of the inoculations. Both worked equally on the feeding of A. africanus. A.J.H. wrote the paper).

(26) HADDOW, A. J., GILLETT, J. D., MAHAFFY, A. F. & HIGHTON, R. B., 1950. "Observations on the biting-habits of some Tabanidae in Uganda, with special reference to arboreal and nocturnal activity." Bull. ent. Res., 41, 209-221. (A.J.H. carried out the main part of the work and wrote the paper. J.D.G. and R.B.H. took part in the wet-season catches of 1944 and A.F.M. in the intensive series of catches in 1945).

(27) HADDOW, A. J., 1950. "A note on the occurrence of Aëdes (Stegomyia) simpsoni Theobald in the canopy of rain-forest in Bwamba County, Uganda". Ann. trop. Med. Parasit., 44, 238-241.

(28) HADDOW, A. J. & van SOMEREN, E. C. C., 1950. "A new species of Stegomyia Theobald from the Ruwenzori Range, Uganda". Ann. trop. Med. Parasit., 44, 281-284.
(A.J.H. discovered the mosquito and contributed the field notes. E. C. C. van S. contributed the actual description).

(29) GILLETT, J. D., ROSS, R. W., DICK, G. W. A., HADDOW, A. J. & HEWITT, L. E., 1950. "Experiments to test the possibility of transovarial transmission of yellow fever virus in the mosquito Aëdes (Stegomyia) africanus Theobald". Ann. trop. Med. Parasit., 44, 342-350.
(A.J.H. initiated this work but thereafter played a small part only as he went on home leave before Experiment 1 had been completed).

(30) SMITHBURN, K. C. & HADDOW, A. J., 1951. "Ntaya virus. A hitherto unknown agent isolated from mosquitoes collected in Uganda". Proc. Soc. exp. Biol. Med., 77, 130-133.
(K.C.S. carried out the laboratory work. A.J.H. collected and identified the mosquitoes).

(31) HADDOW, A. J., van SOMEREN, E. C. C., LUMSDEN, W. H. R., HARPER, J. O. & GILLETT, J. D., 1951. "The mosquitoes of Bwamba County, Uganda. VIII. Records of occurrence, behaviour and habitat". Bull. ent. Res., 42, 207-238.
(A.J.H. contributed a very large proportion of the records of occurrence, behaviour and habitat, and E. C. C. van S. contributed almost all the notes on variation. The others contributed smaller numbers of independent records, in the order shown).

(32) HADDOW, A. J., DICK, G. W. A., LUMSDEN, W. H. R. & SMITHBURN, K. C., 1951. "Monkeys in relation to the epidemiology of yellow fever in Uganda". Trans. R. Soc. trop. Med. Hyg., 45, 189-224.
(A.J.H. collected much of the material and wrote the paper. G.W.A.D. also collected a good deal of material and carried out many of the protection tests. W.H.R.L. contributed most of the West Nile material and some specimens from elsewhere. K.C.S. carried out the bulk of the protection tests).

(33) HADDOW, A. J., 1952a. "Further observations on the biting habits of Tabanidae in Uganda". Bull. ent. Res., 42, 659-674.

(34) HADDOW, A. J., 1952b. "Field and laboratory studies on an African monkey Cercopithecus ascanius schmidti Matschie". Proc. zool. Soc. Lond., 122, 297-394.

(35) HADDOW, A. J., 1952c. "A review of the results of yellow fever protection tests on the sera of Primates from Kenya". Ann. trop. Med. Parasit., 46, 135-143.

(36) DICK, G. W. A., KITCHEN, S. F. & HADDOW, A. J., 1952. "Zika virus. I. Isolations and serological specificity." Trans. R. Soc. trop. Med. Hyg., 46, 509-520.
(G.W.A.D. did almost all the work. S.F.K. made the first isolation from a monkey and A.J.H. contributed the mosquitoes for the second isolation).

(37) DICK, G. W. A. & HADDOW, A. J., 1952. "Uganda S virus. A hitherto unrecorded virus isolated from mosquitoes in Uganda. (I). Isolation and pathogenicity". Trans. R. Soc. trop. Med. Hyg., 46, 600-618.
(Almost entirely G.W.A.D.'s paper. A.J.H. caught and identified the mosquitoes only).

(38) ROSS, R. W., HADDOW, A. J., RAPER, A. B. & TROWELL. H. C., 1953. "A fatal case of yellow fever in a European in Uganda." E. Afr. med. J., 30, 1-11.
(R.W.R. and A.J.H. carried out the field investigation, R.W.R. collecting and testing the human samples, A.J.H. the mosquitoes, and both collecting the monkeys, field history and epidemiological data. A.B.R. carried out the post mortem, R.W.R. the histological work, and A.C.T. was in charge of the case in hospital).

(39) HADDOW, A. J., 1954. "Studies of the biting-habits of African mosquitos. An appraisal of methods employed, with special reference to the twenty-four-hour catch". Bull. ent. Res., 45, 199-242.

(40) HADDOW, A. J., 1956a. "Observations on the biting-habits of African mosquitoes in the genus Eretmapodites Theobald. Bull. ent. Res., 46, 761-772.

(41) HADDOW, A. J., 1956b. "Rhythmic biting activity of certain East African mosquitoes". Nature, Lond., 177, 531-532.

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APPENDIX I

Appendix I - List of full names of insects, animals and plants mentioned in the text

Mosquitoes:

Aëdes (Aëdimorphus) abnormalis Theo.
- argenteopunctatus Theo.
- cumminsi Theo.
- haworthi Edw.
- lamborni Edw.
- mutilus Edw.
- natronius Edw.
- tarsalis Newst.
- simulans N. & C.

(Banksinella) circumluteolus Theo.
- palpalis Newst.
- taeniarostris Theo.

(Diceromyia) furcifer(Edw.)
- taylori(Edw.)

(Finlaya) ingrami Edw.
- longipalpis Grünb.
- pulchrithorax Edw.

(Mucidus) grahami Theo.

(Ochlerotatus) caballus Theo.
- cantans Mg.
- fryeri Theo.

(Skusea) pembaensis Theo.

(Stegomyia) aegypti (L.)
- africanus Theo.
- albopictus (Skuse)
- augustus Edw.
- apicoargenteus Theo.
- bambusae Edw.
- de-boeri Edw.
- de-boeri ssp. de-meilloni Edw.
- dendrophilus Edw.
- fraseri Edw.
- luteocephalus (Newst.)
- metallicus Edw.
- ruwenzori Haddow & van S.
- simpsoni (Theo.)
- vittatus (Big.)

Anopheles (Anopheles) coustani v. ziemanni Grünberg.
- implexus (Theo.)
- neomaculipalpus Curry

(Myzomyia) funestus Giles
- gambiae Giles
- leucosphyrus balabacensis Baisas
- minimus Theo.
- pharoensis Theo.

(Nyssorhynchus) aquasalis Curry

Culex (Culex) antennatus (Becker)
- fatigans Wied.
- musarum Edw.
- sitiens Wied.
- theileri Theo.

(Culiciomyia) nebulosus Theo.

(Lutzia) tigripes Grp. & C.

Eretmapodites chrysogaster Graham
- dracaenae Edw.
- ferox Haddow
- grahami Edw.
- inornatus Newst.
- leucopus ssp. productus Edw.
- oedipodius ssp. parvipluma Edw.

APPENDIX I

Mosquitoes: (cont.)	Eretmapodites penicillatus Edw.
	semisimplicipes Edw.
	silvestris v. conchobius Edw.
	Harpagomyia taeniarostris Theo.
	Megarhinus ruwenzori (van S.)
	Taeniorhynchus (Coquillettidia) aureus Edw.
	aurites Theo.
	fuscopennatus Theo.
	maculipennis Theo.
	metallicus Theo.
	microannulatus Theo.
	pseudoconopas Theo.
	(Mansonioides) africanus (Theo.)
	uniformis (Theo.)
	Toxorhynchites ruwenzori (van S.)
	Uranotaenia yovani van Someren
	ornata v. musarum Edw.
	garnhami van S.
Other Insects:	Aphis fabae Scop.
	Chrysops (Kleineana) centurionis Beg.
	dimidiata Wulp.
	silacea Austen
	Haematopota nefanda Edw.
	Lemurphthirus galagus Bedford
	Liponyssus galagus Zumpt.
	Pegomyia betae (Curt.)
	Simulium ornatum Meigen
	damnosum Theo.
Other invertebrates:	Loa loa (Cobbold)
Vertebrates:	Aquila verreauxi Lesson
	Atelerix pruneri hindei (Thomas)
	Cercocebus albigena johnstoni (Lydekker)
	Cercopithecus aethiops arenarius (Heller)
	centralis Neumann
	johnstoni Pocock
	ascanius schmidti Matschie
	l'hoesti l'hoesti P. L. Sclater
	mitis stuhlmanni Matschie
	neglectus Schlegel
	nictitans mpangae Matschie
	Colobus abyssinicus ituricus Matschie
	matschiei Neumann
	angolensis ruwenzorii Thomas
	polykomos uellensis Matschie
	Dendrohyrax (Heterohyrax) brucei brucei (Gray)
	Erythrocebus patas pyrrhonotus (Hemprich & Ehrenberg)
	Euoticus elegantulus (Le Conte)
	Galago alleni Waterhouse
	crassicaudatus agisymbanus (Coquerel)
	kikuyuensis Lönnberg
	lasiotis Peters
	panganiensis (Matschie)
	senegalensis albipes Dollman
	braccatus Elliot
	senegalensis E. Geoffroy
	zanzibaricus Matschie
	Galagoides demidovii (Fischer)
	thomasi (Elliot)

APPENDIX I - II

Vertebrates: (cont.)
Oryx beisa annectens Hollister
Macaca mulatta Zimm.
Papio doguera (Pulcheran)
Procavia habessinica meneliki Neumann
Rana temporaria L.
Rhinocorax rhipidurus (Hartest)
Rhynchotragus kirkii nyikae Heller
Strepsiceros imberbis australis (Heller)

Plants:
Arundinaria alpina K. Schum.
Canna orientalis Rosc.
Coelomyces africanus Walker
Colocasia esculentum Schott.
Cynometra alexandri C. H. Wright
Dracaena afromontana Mildbr.
fragrans Gawl.
steudneri Schweinf. ex. Engl.
ugandensis Bak.
Elaeis guineensis Jacq.
Ensete edule (Gmelin) Horan
Macaranga schweinfurthii Pax
Maesopsis eminii Engl.
Mitragyna stipulosa (D.C.) O.Ktze.
Musa (Eumusa) acuminata Colla
balbisiana Colla
pardisiaca (L.)
sapientum Kuntze
(Physocaulis) sp. indet. aff. ensete Gmelin
Pandanus chiliocarpus Stapf.
Phoenix reclinata Jacq.
Piptadeniastrum africanum (Hook. f.) Brenan
Pseudospondias microcarpa (A. Rich.) Engl.
Pycnanthus angolensis (Wdw.) Exell.
Raphia monbuttorum Drude
Sansevieria abyssinica N. E. Br.
Xanthosoma sagittifolium Schott.

The author of Rana catesbiana has not been traced up till the time of writing.

Appendix II - Note on Semliki Forest virus and other members of its group

Semliki Forest virus was isolated from mice inoculated with a suspension of A. abnormalis group mosquitoes from Bundinyama. The monkey which received the same suspension did not become sick, but was subsequently found to have become immunised, this showing that it had experienced an infection (Smithburn and Haddow, 1944). A point not mentioned in the paper just cited was that at a later date it was found that not only had this monkey become immune, but that two others, which had been receiving other mosquito groups, had also been immunised. The mice receiving suspensions of these other mosquitoes had remained well, but this is not surprising as the virus, on primary isolation, was of low virulence for mice, though it rapidly became mouse-adapted and exceedingly virulent for these animals. This observation showed that the virus had been present in more than one species of mosquito, and in this connection it is interesting to note that when it was isolated again, years later, at Kumba in the Cameroons, the mosquito host was Eretmapodites grahami, which is not at all closely related to A. abnormalis (Macnamara, 1953).

It is desirable to make a few comments on Semliki Forest virus, as it has proved to be one of great interest. That infections in man are widespread in tropical Africa has been shown by the results of immunity surveys,

APPENDIX II - III

and it is also active far to the south of the tropical belt. It causes severe infections in monkeys and in some other animals, and is one of those interesting viruses which have oncolytic properties (i.e., the property of attacking tumour tissue) as has been shown by Ginder and Friedewald (1951, 1952). Apart from the apparently identical Kumba strain, isolated in West Africa by Macnamara (v.s.) certain other viruses have been discovered recently which are exceedingly closely related to Semliki Forest virus. These are Mayaro virus, isolated in Trinidad from febrile human cases, and Oriboca virus (perhaps identical to Mayaro), isolated in the Oriboca Forest at the mouth of the Amazon from mosquitoes, from "sentinel" monkeys (kept in the forest in the hope that they will become infected with mosquito-borne viruses) and from febrile humans (Dr. Wilbur Downs, Dr. Ottis Causey and Dr. Max Theiler, private communications). There is also evidence that a member of this group is active in Malaya and Borneo (Smithburn, 1954), in India (Smithburn, Kerr and Gatne, 1954) and in South Africa (Kokernot, Smithburn and Weinbren, in press). Another exceedingly interesting virus, very closely allied to Semliki Forest virus, is that of Chikungunya fever, recently isolated in southern Tanganyika by members of the Entebbe Institute staff and colleagues in mission service (Robinson, 1955; Lumsden, 1955a; Ross, 1956; Mason and Haddow, in press). This virus was repeatedly isolated from patients and mosquitoes during a very severe and widespread outbreak of a disease which, from the clinical standpoint, was typical dengue and which, like dengue, was apparently transmitted by A. aegypti. Work in New York, using the haemagglutination technique of Casals and Brown (1954) has, however, shown that Chikungunya virus, like Semliki Forest virus, belongs to Haemagglutination group A, and thus is not closely related to the classical dengue viruses, which belong to Haemagglutination group B. Clinical signs, therefore, can no longer be considered as diagnostic of true dengue fever, and it seems that in future the term "dengue" should be taken as implying a particular clinical syndrome rather than an infection with a particular virus. It may be added that interest in Chikungunya virus is increasing as it has, just prior to the time of writing, been isolated from a human case in the Union of South Africa (Smithburn, private communication) and in the Entebbe area both from mosquitoes (Aëdes africanus) and from a human case (Weinbren, Haddow and Williams, 1956). Semliki Forest virus was thus the first to be isolated of a widely distributed tropical group, which may be of considerable importance in relation to human disease.

The results of the first immunity surveys in humans and wild animals from Kenya and Uganda for antibodies to Semliki Forest virus were reported by Smithburn, Mahaffy and Haddow (1944). In the animal sample immunity to Semliki Forest virus was found only among the Primates, the most heavily affected species being Cercopithecus ascanius schmidti, the redtail monkey. Observations in Bwamba had already shown that this is a monkey of the forest edge, relict forest and second-growth, and that of all the local species it is the most inveterate crop raider. Of 27 of these monkeys tested in the period before the work was written up, 11 (41 per cent) were found to be immune.

Appendix III - Note on Ntaya virus

The catch at Ntaya yielded a very mixed group of mosquitoes, with few suspects where yellow fever was concerned. A group of Eretmapodites spp. and a group of Aëdes (Aëdimorphus) spp. were selected for individual treatment. All the others, a very mixed lot of 1,318 mosquitoes, belonging to five genera, were pooled to economise the use of laboratory animals. In this pool Culex spp. predominated, 1,284 being included. From this lot Ntaya virus was isolated. For detail the original paper (Smithburn and Haddow, 1951) should be consulted.

The mosquitoes were inoculated into mice and a rhesus monkey. The virus was isolated from the mice, and the monkey neither showed objective signs of illness nor became immune. It seemed, therefore, that while the virus might have come from the mosquitoes, it might equally well have been derived from the laboratory mice. Later, however, it was found that this virus is a very poor immunising agent for monkeys, which explained the apparent anomaly.

More recently, immunity surveys have shown that human infections are prevalent in Uganda (Smithburn, 1952a), in Egypt (Smithburn, Taylor, Rizk and Kader, 1954) and in India (Smithburn, Kerr and Gatne, 1954). So far no immune individuals have been found in South Africa (Kokernot, Smithburn and Weinbren, in press). The results of these surveys preclude the possibility that the virus was one derived from the mice, and it may be concluded that it was derived from the Ntaya mosquitoes.

Nothing is known as yet of the clinical picture of the infection in man, and but little is known of the pathogenicity for animals.

Appendix IV - Note on Bunyamwera virus

During September, 1943, a large-scale catch was made on the banks of the Semliki River, in dense, mixed forest, at a point known as Bunyamwera III. It is so named on the Institute maps as there are two other areas called "Bunyamwera" in Bwamba. Altogether 5,345 mosquitoes were taken, and of these 5,133 were sent to Entebbe. Included among these was a lot made up of 4,114 Aëdes, belonging to 14 species, and from these was isolated the agent later named Bunyamwera virus (Smithburn, Haddow and Mahaffy, 1946). It is not possible to say from which of the 14 species this virus was isolated, but it may be noted that two sections of the genus were particularly well represented. Thus there were 1,096 A. tarsalis group and A. abnormalis group, and 2,584 mosquitoes of the subgenus Banksinella, these belonging to three species, A. circumluteolus, A. palpalis and A. taeniarostris. A. palpalis made up over 90 per cent of the total for the subgenus.

This virus may be regarded as an important one as it has been shown by inoculation of human volunteers (cases of inoperable neoplastic disease) that it may cause severe encephalitis with residual mental disturbance of a major order (Southam and Moore, 1951, 1954). It has pronounced oncolytic properties, but has not yet been used effectively in the treatment of tumours and in view of the severe clinical effects which it may produce in man it seems clear that the present unmodified strain is too dangerous for this purpose. As a result of antibody survey, it is known that human cases occur over a wide area in Africa, extending from the Union of South Africa (Kokernot, Smithburn and Weinbren, in press), through East Africa (Smithburn, 1952) to Egypt (Smithburn, Taylor, Rizk and Kader, 1954). It does not appear to be active in India (Smithburn, Kerr and Gatne, 1954).

Some interesting work with reference to this virus was carried out in Bwamba shortly after the isolation. Among 42 monkeys collected in widely distributed areas in the main forest, only one showed immunity to Bunyamwera virus, and this seemed to indicate that, while the virus might occur in the main forest, it was far from common there. The point of special interest was that the immune monkey, a colobus or guereza (Colobus abyssinicus ituricus) was one of a small group collected actually within the mosquito catching area on the banks of the Semliki, during the catch from which the virus was isolated. It thus looked as if the focus of virus activity in the forest might have been strictly localised in the Bunyamwera area. A survey of 298 people resident in Bwamba was also made, five areas being chosen, and 40-70 people from each being tested. Immune individuals were found in all five areas, but the incidence of immunity varied greatly from

APPENDIX IV - V

one to another. Around Buhundu, in the Ruwenzori foothills, the incidence was only 3 per cent, and no immune children were found. In the forest-edge area around Hakitengya the rate was about the same, only 4 per cent. In the opener grasslands and agricultural country around Busaro and Bubandi the rate was higher, about 10 per cent. When, however, the sample from the fifth area was considered, an immunity rate of 25 per cent was found. This area was Rwebisengo on the Semliki River, far out in the grasslands of the Semliki Plains. A point of great interest was that when the catch from which virus was isolated was made at Bunyamwera there was a small illegal fishing settlement of about 12 men hidden in the riverside forest on the Congo shore (which is a closed area) less than half a mile upstream from Bunyamwera. The fishermen in this settlement came from Rwebisengo and there was at that time an illicit traffic in smoked fish between the Bunyamwera reach and Rwebisengo, cargoes being smuggled downstream by canoe. A few months later this small hidden settlement was closed down by the Belgian authorities. In view of the high incidence of immunity found in the Rwebisengo area it seems quite likely that the virus was brought into the forest from the plains by the fishermen. It is interesting to note that one of the mosquito species included in the infected lot, Aëdes circumluteolus, is abundant both in the riverside palm and acacia forest of the Semliki and in the Rwebisengo grasslands and thicket. It is also intersting to note that recently another isolation of Bunyamwera virus has been made, this time in South Africa, in an open area liable to flooding, and thus not unlike Rwebisengo. This new isolation was made from A. circumluteolus (Dr. K. C. Smithburn, private communication).

Appendix V - Note on Rift Valley fever virus

At the time when these isolations were made, the dangerous nature of this virus had not been fully realised. It had been isolated only in Kenya, and after the original studies in the Rift Valley, had disappeared for about 14 years.

In 1930 there was a destructive outbreak of a hitherto undescribed virus disease among sheep and cattle near Naivasha, in the Eastern Rift Valley, where it passes through Kenya. Mortality was very high. A few human cases also occurred, but these were not serious, a mild dengue-like fever being the usual clinical picture. The virus was isolated by Daubney and Hudson (1931), who concluded that the vector was a mosquito, "Taeniorhynchus brevipalpis" (now known as T. fuscopennatus). The virus causes extensive liver damage, and is the only liver-destroying virus so far known which can be maintained easily in an inexpensive laboratory animal, the white mouse (there is no known animal host for serum jaundice or infective hepatitis, and in the case of yellow fever, which attacks the liver in monkeys and man, the virus becomes neurotropic in mice, and attacks the central nervous system). Thus Rift Valley fever is of great importance in laboratory virus work. It has, however, gained a very bad reputation as workers with this virus almost all undergo an infection, sometimes severe, and for this reason (and also in the fear that cattle or sheep may become infected) many countries, such as U.S.A. and Australia, will not permit its entry.

In the catch which yielded yellow fever virus, carried out between 18th and 22nd April, there were separate inoculation lots of Eretmapodites spp. and A. tarsalis group, and both of these lots yielded Rift Valley fever virus. The next large routine catch at Mongiro was from 16th to 20th May, and once again two strains of this virus were obtained, one from Eretmapodites spp. and one from a lot of A. dendrophilus (at that time listed, as explained above, as A. de-boeri ssp. de-meilloni). Another catch took place between 23rd and 27th May, and once again two strains were recovered, one from Eretmapodites spp. and one from A. tarsalis group. Thus there were three isolations from Eretmapodites spp. (the lots included the E. chrysogaster group, E. inornatus group, E. ferox and E. leucopus ssp. productus), two from the A. tarsalis group, and one from A. dendrophilus. No cases of infection are known to have occurred among the mosquito catchers. It is interesting to note that during this work a dead buffalo was found actually within the catching area, and a sick buffalo calf was seen several times. There was no obvious outbreak of illness among local native stock. These isolations have been reported in detail by Smithburn, Haddow and Gillett (1948).

Following the isolations, tests for immunity to this virus were carried out on the sera of 72 Bwamba monkeys, belonging to nine species. Fifteen of these came from Mongiro, ten having been collected there during or after the time of the virus isolations. There were no immune specimens in this sample. The reason appears to be that, as was shown by the platform catches, all the mosquitoes from which virus was isolated bite preferentially at ground level, being scarce in the trees. A. dendrophilus is in fact, poorly named. It is the only known Uganda species of the subgenus Stegomyia which is not to some extent dendrophilic.

Subsequently the virus was successfully transmitted by the bite of mosquitoes of the E. chrysogaster group from mouse to mouse, from mouse to lamb, from lamb to lamb and from lamb to mouse (Smithburn, Haddow and Lumsden, 1949b). Since that time a great deal of laboratory work has been carried out with Rift Valley fever virus at Entebbe. There have been 18 cases of Rift Valley fever among the workers at the Institute and, though none have been fatal, some have been very severe. Some of the earlier cases have been described by Smithburn, Mahaffy, Haddow, Kitchen and Smith (1949).

The full importance of this virus was not realised till some years later, when an extensive epidemic occurred in the Union of South Africa (Mundel and Gear, 1951; Joubert, Ferguson and Gear, 1951; Gear, de Meillon, Measroch and Harwin, 1951). Not only was there widespread destruction of stock, but many human cases occurred. In a certain number of the latter retinal damage during the illness was followed by more-or-less serious visual defects. The vaccine finally developed at Onderstepoort to combat this epidemic was made from a high-passage neurotropic line of virus evolved at Entebbe by brain-to-brain passage in mice of one of the Bwamba strains. It is interesting to note that the entomological investigations showed that in South Africa the disease could not have been carried by any of the mosquitoes involved in the Bwamba isolations, which in their turn differed from the species thought to be the vector in the original outbreak at Naivasha. In a still later epidemic in the Orange Free State, virus was isolated from Culex theileri and Aëdes caballus (Gear, de Meillon, Roux and five others, 1955), and very recently Smithburn, working in Zululand, has made several isolations from A. circumluteolus (private communication). At the time of writing two new strains have been isolated near Entebbe from A. circumluteolus and A. africanus (Weinbren, Williams and Haddow, 1956), and it thus seems obvious that this important and most dangerous virus can be transmitted by a number of species, belonging to different genera, by far the majority of the isolations having been made from species which bite mainly at ground-level. In this connection it should be mentioned that the isolation from A. africanus just referred to was made in an area where there is no continuous canopy, merely scattered residual clumps of trees rising from dense bush. Here quite unusually large numbers of A. africanus are to be found at ground level, as by day they seem unable to remain in the sparse, discontinuous canopy, much exposed as it is to sun and wind.

Appendix VI- Notes on the biting cycle of Chrysops centurionis and on its relationship to the distribution of loiasis

When analysis was begun on the sample of C. centurionis obtained in the

Bwamba Tree Survey it was found that, as in certain mosquitoes, the form of the biting cycle was influenced by the time at which biting began. When the first biting activity occurred early - before 17 hours - the peak was ill-defined, and spread over a period of about 3 hours. When it began in the period 17-18 hours the peak was more clearly defined, and covered a two-hour period. When it began in the period 18-19 hours, it was sharply defined, and virtually confined to this single hour. When biting began after 19 hours the peak was ill-defined, but this was compensated by a higher level of activity than usual during the rest of the night. In the first three cases the biting-peak made up a very constant proportion of the entire yield (63, 71 and 64 per cent respectively) and this was taken as suggesting that a particular section of the population might be active at this time (this idea had previously been advanced by Mattingly, 1949a and by Lumsden, 1952 in an attempt to explain the behaviour of certain mosquitoes). In the last group, when biting began late, the peak represented only 49 per cent of the whole yield. These were, however, the unfavourable days, and it is felt that in many cases the peak may have failed to develop, owing to adverse weather conditions. Further analysis showed that the highest yield was on days when biting began early, and that the later it began the smaller was the catch. Finally it was found that the shorter the total spell of activity, the more intense was the activity recorded.

It may be mentioned that during these years much field work was being carried out in other parts of Uganda (see above) and that this work confirmed that everywhere C. centurionis was found its biting habits were arboreal and crepuscular. For details of the studies on this Tabanid the published work (Haddow, 1952b) should be consulted. This paper also contains notes on a number of other Uganda Tabanids, and it is pointed out that almost half of them show evidence of crepuscular activity. The biting-cycle of Haematopota nefanda is also discussed, this being of the eo-crupuscular type, with peaks in the sunset and sunrise periods.

In the same paper is discussed the question of transmission of Loa loa by Tabanids, and it is pointed out that here is a particularly satisfactory example of the epidemiology and distribution of a human disease being dependent on the biting-habits of the insect vector. Gordon, Kershaw, Crewe and Oldroyd (1950) showed that the vectors of loiasis, C. silacea and C. dimidiata are, like C. centurionis, mainly arboreal. They, however, readily descend to attack man, which C. centurionis will not do. The distribution of loiasis in man agrees closely with that of these two species, and the disease stops in the eastern Belgian Congo, just where these Tabanids are replaced by C. centurionis. The other very important point is that whereas C. silacea and C. dimidiata are essentially diurnal, C. centurionis is crepuscular, biting at a time when Africans do not normally enter the forest. This species may well, however, maintain loiasis among the monkeys, and it is to be noted that Gordon and his co-workers (loc.cit.) suggest that monkeys may be involved in the epidemiology, acting as the main hosts where man is absent or scarce. Even in the case of monkeys, however, C. centurionis is probably a less satisfactory vector of this particular filaria than are the other two, as the periodicity of the microfilaria in the peripheral circulation is essentially diurnal. In the case of the Dirofilaria sp. which is common in East African monkeys, it may be more efficient, and is probably the usual vector.

Appendix VII - Note on the Mengo strains of encephalomyocarditis virus

In June, 1946, an unused rhesus monkey in one of the open-air monkey runs at the Institute became sick, and from it was isolated a virus which was thought to be new and which was named Mengo encephalomyelitis virus. Later this was found to be a strain of a known virus, that of encephalomyocarditis (usually known as EMC) which has the property of damaging both heart muscle and the central nervous system.

Subsequently the virus was isolated from Taeniorhynchus fuscopennatus collected around the run in which the monkey had acquired its infection, from a mixed lot of Taeniorhynchus spp. taken at Zika during the course of the Entebbe Tree Survey, and from a sick mongoose trapped in the Institute compound, near the run where the original infection occurred. Somewhat later an isolation was made from a human case. This work has been described elsewhere (Dick, Smithburn and Haddow, 1948; Dick, Best, Haddow and Smithburn, 1948).

In 1950, the virus having been recovered twice again from captive monkeys at Entebbe, large scale catches were begun in the Institute compound by the writer, 36,638 mosquitoes being taken. Dick carried out the inoculations, and in all four isolations of this virus were made from T. fuscopennatus and one from T. uniformis. Subsequently, however, it has been shown that the virus is not transmitted by T. fuscopennatus, though it may persist in this mosquito for some time. It is thought that T. fuscopennatus probably picks up the virus from the natural hosts, wild rats, or from monkeys which have been infected by them (Kilham, Mason and Davies, 1956a).

The Mengo strains have been of particular interest on account of the rapid destruction which they cause in the heart muscle of certain wild rats and of viverrids, and a good deal of pathological work on this agent is in progress at Entebbe. Young mongooses, for example, may die of fulminating myocarditis only 50-60 hours after inoculation with the virus (Kilham, Mason and Davies, 1956b; Weinbren, 1956).

Appendix VIII - Note on Zika virus

This agent was first isolated in April, 1947, from a febrile sentinel rhesus monkey stationed on one of the Zika platforms, and subsequently from a lot of A. africanus taken during the Entebbe Tree Survey at Zika No. III station (Dick, Kitchen and Haddow, 1952). Not much is known yet about Zika virus. It may be mentioned, however, that immunity in man has been found in various localities in East Africa (Smithburn, 1952a), in Egypt (Smithburn, Taylor, Rizk and Kader, 1954) and in India (Smithburn, Kerr and Gatne, 1954).

The first isolations from man were made in Nigeria, the virus being obtained from three febrile patients during an outbreak of jaundice (Macnamara, 1954). There seemed to be evidence that Zika virus was the cause of the outbreak, but a subsequent experimental infection in a volunteer caused merely a short febrile illness without jaundice (Bearcroft, 1956).

Just before the time of writing two further isolations from mosquitoes have been made at Lunyo near Entebbe. In both cases the mosquitoes were A. africanus. This work was carried out by Dr. M. P. Weinbren and Dr. M. C. Williams. Since these isolations Weinbren, who is carrying out histopathological work with the new strains, has found that Zika virus causes damage to heart muscle in laboratory mice.

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HIGHER DEGREES AND SPECIAL STUDY AND RESEARCH

GENERAL INFORMATION

Higher Degrees

The University awards the higher degrees of Doctor of Letters (D.Litt.) and Doctor of Music (D.Mus.) in the Faculty of Arts, Doctor of Medicine (M.D.), Master of Surgery (Ch.M.) and Master of Dental Surgery (M.D.S.) in the Faculty of Medicine, Doctor of Science (D.Sc.) in the Faculties of Science and Engineering, and Doctor of Philosophy (Ph.D.) in all Faculties.[1]

The regulations governing these degrees are printed below. Candidates for any higher degree are required to submit a thesis embodying original work ; candidates for the degrees of Doctor of Medicine, Master of Surgery and Doctor of Music are also required to submit to examination and candidates for any other higher degree may be required to do so. For the degrees of Doctor of Medicine and Master of Surgery only Bachelors of Medicine of this University, and for the degree of Doctor of Science in Public Health only Bachelors of Science in Public Health of this University, may be candidates, and no residence is required for these degrees. The degree of Master of Dental Surgery is open to Bachelors of Dental Surgery of this University, and to Bachelors of Medicine or of Science of this University who hold a registrable dental qualification. The degrees of Doctor of Letters and Doctor of Science are open both to graduates in Arts or Pure Science or Engineering with Honours of this University, of whom no further residence is required, and to other graduates of this University, or graduates of other Universities, who have spent a prescribed period as Research Students in this University. The degree of Doctor of Music is open both to Bachelors of Music with Honours of this University, of whom no further residence is required, and to Bachelors of Music of other Universities who have spent a prescribed period as Research Students in this University. The degree of Doctor of Philosophy is open to graduates of this University or of other Universities ; all candidates are required to spend a prescribed period as Research Students in this University.[2]

The conditions under which candidates are admitted to the status of Research Student are printed below, pp. 505 *et seq.* The basic fee payable by Research Students, in addition to the normal matriculation fee of £2 12s. 6d. a year, is £9 9s. a year with a supplementary fee of £12 12s. a year where laboratory facilities are given.

Enquiries regarding admission to the status of Research Student should be addressed to the Clerk of Senate.

Diplomas and Certificates for Post-graduate Study

Graduates and other advanced students may be candidates for the Diploma and the Certificate of Proficiency awarded for special study in the Faculty of Arts. The Diploma is intended for Honours graduates who have pursued advanced study of a special subject under the direction of a Professor or Lecturer, the Certificate for others who have attended one of the regular advanced courses provided in the Faculty. The minimum period of study required either for the Diploma or for the Certificate is one year ; in addition to the normal matriculation fee, candidates are required to pay a tuition fee of seven guineas a year and an examination fee of three guineas. The regulations are printed in the syllabus of the Faculty of Arts.

[1] The Regulations for the Degree of Bachelor of Letters appear in the Syllabus of the Faculty of Arts. [2] An Ordinance instituting a Higher Degree in Law is at present being made.

The Regulations for the Diploma in Public Health are printed in the syllabus of the Faculty of Medicine.

I. DEGREE OF DOCTOR OF LETTERS

The Degree of Doctor of Letters is awarded under Ordinance XXVI (Glasgow No. 7), which came into force on 26th September, 1908. The following are the relevant provisions of the Ordinance.

VII. Graduates who have obtained any degree in the University of Glasgow, and who have either before or after graduation passed the Examination in an Honours Group for the Degree in Arts, or the Final Science Examination for the degree in Pure Science or in Engineering with Honours may offer themselves for the degree of Doctor of Letters (D.Litt.) after the expiry of five years from the date of their graduation.

VIII. Research Students as aforesaid, who have prosecuted in the University of Glasgow some special study under Ordinance No. 61 (General, No. 23—Regulations for the Encouragement of Special Study and Research, and for the appointment of Research Fellows), may offer themselves for the degree of Doctor of Letters ; provided—

(1) That they have obtained a degree in any Scottish University, or a degree in another University specially recognised by the University Court for the purpose of this section, which the Senatus shall deem to be equivalent to the corresponding degree in the University of Glasgow ; and provided that candidates who have obtained any such degree in a University outside the United Kingdom so recognised may be required if the Senatus think fit, before beginning their course as Research Students with a view to the degree of Doctor of Letters, to pass an examination equivalent to an Honours Examination in a group of subjects cognate to their line of work as Research Students.

(2) That they have spent not less than two terms in each of two academical years, or an equivalent period, as Research Students in the University of Glasgow, and produce to the Senatus evidence of satisfactory progress in the special study undertaken by them during that period.

(3) That a period of not less than five years shall have elapsed from the date of the graduation required in sub-section (1) of this section.

IX. All candidates for the degree of Doctor of Letters shall present a thesis or a published memoir or work, which shall be an original contribution to learning in relation to literature or to philosophy, to be approved by the Senatus on the recommendation of a Special Committee appointed by the Senatus ; provided that, if required by the Senatus, a candidate shall also be bound to pass such an examination, conducted orally or otherwise, on the subjects of his special study, or his thesis, or memoir or work, as may from time to time be determined. The thesis or memoir or work shall be accompanied by a declaration signed by the candidate that it has been composed by himself. If the thesis has not already been published, it shall be published by the candidate in such a manner as the Senatus shall approve, and a copy thereof shall be deposited by the candidate in the University Library.

X. Notwithstanding, and in supplement of the provisions of Ordinance No. 13 (General, No. 8—Regulations as to Examinations), Sections

XIV and XV, the Senatus shall appoint such Professors or Lecturers in the University as it may think suitable to examine the theses and to conduct the examination of candidates who may offer themselves under the provisions of Ordinance No. 12 (General, No. 7, Regulations for Degrees in Science) or of Ordinance No. 23, Glasgow No. 2—Regulations for Degrees in Engineering, Science or of this Ordinance, for the degree of Doctor of Letters ... ; and the University Court shall, after consultation with the Senatus, appoint one or more additional Examiners to act along with them in adjudicating on the merits of the candidates. Every such additional Examiner shall be a person of recognised eminence in the subject of the thesis or memoir or work which is to be submitted for approval, and may be a Professor or Lecturer in any Scottish University other than the University of Glasgow. The result of the examination and adjudication shall be reported to the appropriate Faculty or Special Committee of Senatus, who shall if they think fit make a recommendation thereon to the Senatus ; and no candidate shall be approved for the degree unless the Senatus is satisfied that his work is of distinction as a record of original research undertaken by himself, or of important engineering work designed by himself and actually carried out, or as an original contribution to learning.

SUPPLEMENTARY REGULATIONS

1. The thesis should be presented in the form of a single memoir or writing containing a connected account of the candidate's research or work. Detached papers under various headings will not be regarded as a sufficient substitute, unless they are accompanied by a separate statement, composed by the candidate, giving a full account of the methods, results and conclusions of the research or work on which his candidature is based.

Along with this thesis each candidate must submit a résumé of it, stating what is claimed as original in it, and a bibliography.

2. Candidates who have been awarded a Certificate of Proficiency in the subjects of an Honours Group will be regarded as having "passed the Examination in an Honours Group" for the purpose of proceeding to the degree of D.Litt.

3. Each candidate must submit two copies of his thesis : both copies, whether the thesis is approved for the degree or not, shall become the property of the University.

2. DEGREE OF DOCTOR OF MUSIC

The Degree of Doctor of Music was instituted by Ordinance CLXXIII (Glasgow No. 42) ; the following are the sections of that Ordinance which govern the award of the Degree.

XI. (1) Bachelors of Music of the University of Glasgow, who have taken Honours either before or after graduation, may offer themselves for the degree of Doctor of Music (D.Mus.), after the expiry of five years from the date of their graduation.

(2) Bachelors of Music of other Universities recognised for the purpose by the University Court after consultation with the Senatus may offer themselves for the degree of Doctor of Music, after the expiry of

five years from the date of their graduation, provided they have spent not less than three years as Research Students in the University of Glasgow, under Ordinance No. 61 (General, No. 23), and produce to the Senatus evidence of satisfactory progress in the special study undertaken by them during that period.

XII. The Degree shall be given in three Departments, and candidates may present themselves in one or more of these Departments.

The Departments shall be those of:

(*a*) Composers;
(*b*) Executants;
(*c*) Theorists or Historians.

Composers

XIII. (1) Candidates for the Degree of Doctor of Music as Composers shall submit a prescribed number of original works in accordance with regulations to be prescribed by the Senatus.

Compositions must be accompanied by a declaration signed by the candidate that they are his own unaided work, and that no portion has been submitted previously to any University.

(2) Candidates in this department shall also be examined in the following subjects:

(*a*) Eight-part Harmony and Counterpoint;
(*b*) Canon and Double Counterpoint in four parts, and Fugue in five parts;
(*c*) Scoring for full Orchestra;
(*d*) Historical Knowledge.

Executants

XIV. (1) Candidates for the Degree of Doctor of Music as Executants shall be required to pass a test of a wide repertoire of concert works in accordance with regulations to be prescribed by the Senatus.

(2) Candidates in this department must also qualify for the optional subject set forth in VI (8) p. 276 (Degree of Bachelor of Music), and may also be required to pass an examination in any or all of the subjects prescribed for candidates for the Degree of Doctor of Music as Composers in accordance with regulations to be prescribed by the Senatus.

Theorists or Historians

XV. (1) Candidates for the Degree of Doctor of Music as Theorists or Historians shall present, in accordance with regulations to be prescribed by the Senatus, one or more treatise on Theoretical or Historical subjects. Such treatises must be the result of original thought and research, not merely abstracts or compilations of existing works.

Each treatise must be accompanied by a declaration signed by the candidate that it is his own unaided work and that it has not been submitted to any other University.

(2) Candidates in this department may also be required to pass an examination in any or all of the subjects prescribed for candidates for the Degree of Doctor of Music as Composers, in accordance with regulations to be prescribed by the Senatus.

SUPPLEMENTARY REGULATIONS

1. **Executants, Theorists and Historians.** All candidates in these categories will be examined in the following four subjects :

(*a*) Harmony and counterpoint in not more than eight parts.

(*b*) Canon and Double and Triple counterpoint in not more than three parts, and Fugue in not more than five parts.

(*c*) Scoring for full orchestra.

(*d*) The History of music from 1500 A.D. to the present day.

2. **Executants.** Each candidate must submit, not later than six weeks before the examination, an extensive list of works which he professes ; if the list is approved, he will be informed, a month before the examination, of not more than four works which he will be required to perform. Violinists and violoncellists must include one or more of the unaccompanied sonatas or suites of J. S. Bach ; candidates who profess instruments other than pianoforte, violin, violoncello or organ must include concertos and chamber works ; vocalists must include rôles in opera and oratorio.

3. **Theorists and Historians.** Before submitting a treatise, candidates must submit a précis indicating its scope and general character ; a treatise may not be submitted until the précis has been approved.

3. DEGREE OF DOCTOR OF MEDICINE

The following regulations for the award of the degree of Doctor of Medicine are contained in Ordinance XXXI (Glasgow No. 9).

XXII. (1) Subject to the conditions hereinafter specified, the Degree of Doctor of Medicine may be conferred on any candidate who has obtained the Degrees of Bachelor of Medicine and Bachelor of Surgery of the University of Glasgow, and is of the age of twenty-four years or upwards, and has produced a certificate showing that, after having received the degrees of Bachelor of Medicine and Bachelor of Surgery, he has been engaged for at least one year in attending the Medical Wards of a Hospital or in scientific work bearing directly on his profession, such as is conducted in the Research Laboratories of the University, or in the Naval, Military, Colonial, or Public Health Medical Services, or has been engaged for at least two years in Practice other than Practice restricted to Surgery.

(2) Each candidate for the degree of Doctor of Medicine shall be required to pass an examination in Clinical Medicine or in such special department of Medical Science or Practice professed by the candidate as the Senatus, on the recommendation of the Faculty of Medicine, may approve ; and he may be admitted to the examination at such time, not sooner than one year after he has received the degrees of Bachelor of Medicine and Bachelor of Surgery, as the Senatus may appoint for the purpose.

(3) Each candidate for the degree of Doctor of Medicine shall submit for the approval of the Faculty of Medicine a thesis on any branch of knowledge, comprised in the several divisions of the Examination for the degrees of Bachelor of Medicine and Bachelor of Surgery, which he

may have made a subject of special study, excepting a subject that is exclusively surgical ; and the thesis, accompanied by a declaration signed by the candidate that the work has been done and the thesis composed by himself, shall be lodged with the Dean of the Faculty of Medicine on or before a date to be fixed by the Senatus. The Faculty may, if it sees fit, before approving the thesis, require the candidate to present himself for oral or other examination on the subject-matter thereof. If the thesis is, in the judgment of the Faculty, of special merit, the Senatus may, on the recommendation of the Faculty, exempt the candidate from the whole or part of the examination prescribed in sub-section 2 of this Section.

(4) A Bachelor of Medicine and Bachelor of Surgery, who produces to the Senatus satisfactory evidence of his intention of entering within twelve months after obtaining such degrees on the practice of his profession in a British Possession or Colony, or in a Foreign Country, may, under such conditions as the Senatus may from time to time prescribe, be admitted to the examination in Clinical Medicine or in a special department of Medical Science or Practice prescribed in sub-section 2 of this Section at such time after he has received such degrees as the Senatus may appoint for the purpose : provided always that in special circumstances the Senatus may, if it sees fit, on the recommendation of the Faculty of Medicine, exempt him from the whole or part of the Examination ; but the degree of Doctor of Medicine shall not be conferred on him unless he shall produce a certificate showing that, after having received the degrees of Bachelor of Medicine and Bachelor of Surgery, he has been engaged for at least one year in attending the Medical Wards of a Hospital or in scientific work bearing directly on his profession, such as is conducted in the Research Laboratories of the University, or in the Naval, Military, Colonial or Public Health Medical Services, or for at least two years in Practice other than Practice restricted to Surgery, and unless his thesis, in the judgment of the Faculty of Medicine, is of special merit.

SUPPLEMENTARY REGULATIONS

1. To comply with the conditions prescribed in sub-sections 2 and 3 above, a candidate, after submitting a thesis, may be required to present himself before the examiners for interview or for further examination on the subject-matter of his thesis and related subjects. When a candidate is required to undergo further examination, this examination may be a written, or oral, or practical test, or any combination of these, as the examiners think fit.

2. One copy only of a thesis is required. It must be typewritten on paper of crown quarto size (10 inches by 7½ inches), bound in cloth with stiff boards, and have its title and the name of the author printed in block letters on the outside binding. The thesis should be lodged with the Dean of the Faculty of Medicine not later than 15th September, or 15th December, or 15th March, for adjudication during the Martinmas, Candlemas, and Whitsun terms respectively.

3. A thesis will not be approved unless it gives evidence of original observation, or, if it deals with the researches of others, gives a full statement of the literature of its subject with accurate references and critical investigation of the views or facts cited : mere compilations will in no case be accepted.

4. A thesis submitted for the degree must be a dissertation written for the purpose, provided that the results of original observations already published in medical or scientific journals or in the transactions of learned societies or otherwise may be accepted in place of such a dissertation.

5. Three grades of distinction may be awarded for the excellence of theses submitted for the degree—Commendation, High Commendation and Honours.

6. The copies of theses submitted by candidates, whether the theses are approved for the degree or not, shall become the property of the University.

4. DEGREE OF MASTER OF SURGERY

The following regulations for the award of the degree of Master of Surgery are contained in Ordinance XXXI (Glasgow No. 9).

XXIII. (1) Subject to the conditions hereinafter specified the degree of Master of Surgery may be conferred on any candidate who has obtained the degrees of Bachelor of Medicine and Bachelor of Surgery of the University of Glasgow, and is of the age of twenty-four years or upwards, and has produced a certificate showing that, after having received the degrees of a Bachelor of Medicine and Bachelor of Surgery, he has been engaged for at least one year in attending the Surgical Wards of a Hospital or in scientific work bearing directly on his profession, such as is conducted in the Research Laboratories of the University, or in the Naval, Military, or Colonial Medical Services, or has been engaged for at least two years in Practice other than Practice restricted to Medicine.

(2) Each candidate for the degree of Master of Surgery shall be required to pass an examination in the following subjects: Surgical Anatomy, Operations upon the dead body, and Clinical Surgery or such special department of Surgery professed by the candidate as the Senatus, on the recommendation of the Faculty of Medicine, may approve; and he may be admitted to the examination at such time, not sooner than one year after he has received the degrees of Bachelor of Medicine and Bachelor of Surgery, as the Senatus may appoint for the purpose.

(3) Each candidate for the degree of Master of Surgery shall submit for the approval of the Faculty of Medicine a thesis on any branch of knowledge, comprised in the several divisions of the Examination for the degrees of Bachelor of Medicine and Bachelor of Surgery, which he may have made a subject of special study, excepting a subject that is exclusively medical; and the thesis, accompanied by a declaration signed by the candidate that the work has been done and the thesis composed by himself, shall be lodged with the Dean of the Faculty of Medicine on or before a date to be fixed by the Senatus. The Faculty may, if it sees fit, before approving the thesis, require the candidate to present himself for oral or other examination on the subject-matter thereof. If the thesis is, in the judgment of the Faculty, of special merit,

the Senatus may, on the recommendation of the Faculty, exempt the candidate from the whole or part of the examination prescribed in sub-section 2 of this Section.

(4) A Bachelor of Medicine and Bachelor of Surgery who produces to the Senatus satisfactory evidence of his intention of entering within twelve months after obtaining such degrees on the practice of his profession in a British Possession or Colony, or in a Foreign Country, may, under such conditions as the Senatus may from time to time prescribe, be admitted to the examination in the subjects specified in sub-section 2 of this Section, at such time after he has received such degrees as the Senatus may appoint for the purpose : provided always that in special circumstances the Senatus may, if it sees fit, on the recommendation of the Faculty of Medicine, exempt him from the whole or part of the examination ; but the degree of Master of Surgery shall not be conferred on him unless he shall produce a certificate showing that, after having received the degrees of Bachelor of Medicine and Bachelor of Surgery, he has been engaged for at least one year in attending the Surgical Wards of a Hospital or in scientific work bearing directly on his profession, such as is conducted in the Research Laboratories of the University, or in the Naval, Military, or Colonial Medical Services, or for at least two years in Practice other than Practice restricted to Medicine, and unless his thesis in the judgment of the Faculty of Medicine is of special merit.

SUPPLEMENTARY REGULATIONS

1. To comply with the conditions prescribed in sub-sections 2 and 3 above, a candidate, after submitting a thesis, may be required to present himself before the examiners for interview or for further examination on the subject-matter of his thesis and related subjects. When a candidate is required to undergo further examination, this examination may be a written, or oral, or practical test, or any combination of these, as the examiners think fit.

2. One copy only of a thesis is required. It must be typewritten on paper of crown quarto size (10 inches by 7½ inches), bound in cloth with stiff boards, and have its title and the name of the author printed in block letters on the outside binding. The thesis should be lodged with the Dean of the Faculty of Medicine not later than 15th September, or 15th December, or 15th March, for adjudication during the Martinmas, Candlemas, and Whitsun Terms respectively.

3. A thesis will not be approved unless it gives evidence of original observation, or, if it deals with the researches of others, gives a full statement of the literature of its subject with accurate references and critical investigation of the views or facts cited : mere compilations will in no case be accepted.

4. A thesis submitted for the degree must be a dissertation written for the purpose, provided that the results of original observations already published in medical or scientific journals or in the transactions of learned societies or otherwise may be accepted in place of such a dissertation.

5. Three grades of distinction may be awarded for the excellence of theses submitted for the degree—Commendation, High Commendation and Honours.

6. The copies of theses submitted by candidates, whether the theses are approved for the degree or not, shall become the property of the University.

5. DEGREE OF DOCTOR OF SCIENCE

The degree of Doctor of Science is awarded under Ordinance XXVI (Glasgow No. 7), which came into force in September, 1908. The following are the relevant provisions of that Ordinance, with the amendments made in subsequent Ordinances.

I. Graduates who have obtained any degree in the University of Glasgow, and who have either before or after graduation passed the Examination in an Honours Group for the degree in Arts, or the Final Science Examination for the degree in Pure Science or in Engineering with Honours, may offer themselves for the degree of Doctor of Science (D.Sc.) after the expiry of five years from the date of their graduation.

II. Research Students within the meaning of Ordinance No. 61 (General No. 23—Regulations for the Encouragement of Special Study and Research and for the Appointment of Research Fellows), who have prosecuted in the University of Glasgow (or in a College affiliated thereto) some special study or research under that Ordinance, may offer themselves for the degree of Doctor of Science : provided :

(1) That they have obtained a degree in any Scottish University, or a degree in another University specially recognised by the University Court for the purpose of this section which the Senatus shall deem to be equivalent to the corresponding degree in the University of Glasgow ; and provided that candidates who have obtained any such degree in a University outside the United Kingdom so recognised may be required, if the Senatus think fit, before beginning their course as Research Students with a view to the degree of Doctor of Science, to pass an examination equivalent to an Honours or to a Final Science Examination in a group of subjects cognate to their line of work as Research Students.

(2) That they have spent not less than two terms in each of two academical years, or an equivalent period, as Research Students in the University of Glasgow (or in a College affiliated thereto), and that they produce to the Senatus evidence of satisfactory progress in the special study or research undertaken by them during that period.

(3) That a period of not less than five years shall have elapsed from the date of the graduation required in subsection (1) of this section.

III. All candidates for the degree of Doctor of Science shall present a thesis or a published memoir or work, to be approved by the Senatus on the recommendation of the Faculty of Science ; provided that, if required by the Senatus, the candidate shall also be bound to pass such an examination conducted orally or practically, or by written papers, or by all of these methods, on the subjects of his special study or of his thesis, memoir, or work, as may from time to time be determined. The thesis shall be a record of original research in relation to science undertaken by the candidate, or of some important engineering work designed by the candidate and actually carried out, and shall be accompanied by a declaration signed by him that the work has been done and the thesis composed by himself. If the thesis has not already been published, it shall be published by the candidate in such manner as the Senatus shall approve, and a copy thereof shall be deposited by the candidate in the University Library.

X. Notwithstanding, and in supplement of the provisions of Ordinance No. 13 (General, No. 8—Regulations as to Examinations), Sections XIV and XV, the Senatus shall appoint such Professors or Lecturers in the University as it may think suitable to examine the theses and to conduct the examination of candidates who may offer themselves under the provisions of Ordinance No. 12 (General, No. 7—Regulations for Degrees in Science) or of Ordinance No. 23 Glasgow, No. 2—Regulations for Degrees in Engineering Science, or of this Ordinance, for the degree of Doctor of Science . . . ; and the University Court shall, after consultation with the Senatus, appoint one or more additional Examiners to act along with them in adjudicating on the merits of the candidates. Every such additional Examiner shall be a person of recognised eminence in the subject of the thesis or memoir or work which is to be submitted for approval, and may be a Professor or Lecturer in any Scottish University other than the University of Glasgow. The result of the examination and adjudication shall be reported to the appropriate Faculty or Special Committee of Senatus, who shall if they think fit make a recommendation thereon to the Senatus ; and no candidate shall be approved for the degree unless the Senatus is satisfied that his work is of distinction as a record of original research undertaken by himself, or of important engineering work designed by himself and actually carried out, or as an original contribution to learning.

SUPPLEMENTARY REGULATIONS

1. Candidates who have been awarded a Certificate of Proficiency in the subjects of an Honours Group will be regarded as having "passed the Examination in an Honours Group" for the purpose of proceeding to the degree of D.Sc.

2. Each candidate must submit two copies of his thesis : both copies, whether the thesis is approved for the degree or not, shall become the property of the University.

3. In addition to making the declaration required under Section III of the Ordinance a candidate must, if the whole or any part of the subject-matter of the thesis submitted by him has been included in a thesis already approved for a degree in this or another University, make a declaration to that effect, and must lodge together with his thesis either a copy of such previously approved thesis or a precise statement of its scope.

4. Before acceptance for adjudication, a thesis, or an important part of it, shall have been published either as a book or in periodicals of recognised standing. The thesis may be presented in the form of a single memoir or writing containing a connected account of the

candidate's research or work. Published papers under various headings may be submitted in lieu of a single thesis provided that they are accompanied by a statement showing the relationship between the various studies and placing the whole work critically into perspective with the general state of knowledge in the field of investigation to which the candidate's researches are related. The thesis should also be accompanied by two copies of a separate summary (500–1000 words) which must be an adequate and informative abstract of the work, suitable for publication by the University.

6. DEGREE OF DOCTOR OF SCIENCE IN PUBLIC HEALTH

The regulations for the award of the degree of Doctor of Science in Public Health are contained in Ordinance VI (Glasgow No. 2), which came into force in May, 1903. The relevant provisions of the Ordinance are :

X. Graduates who have held the degree of Bachelor of Science in Public Health from the University of Glasgow for a term of five years, may offer themselves for the degree of Doctor of Science in Public Health in the said University.

XI. Each candidate for the degree of Doctor of Science in Public Health shall present a thesis or a published memoir or work to be approved by the Senatus, on the recommendation of the Faculty of Science, and shall also be required to pass an examination in Public Health, and in such of its special departments as the Senatus, with the approval of the University Court, by regulations framed from time to time, shall determine.

The thesis, or published memoir or work, shall be a record of original research undertaken by the candidate, and shall be accompanied by a declaration, signed by him, that the work has been done, and the thesis or memoir composed, by himself.

XII. The Senatus Academicus shall appoint such Professors or Lecturers as it may think suitable to conduct the examination of candidates who may offer themselves under the provisions of this Ordinance for the degree of Doctor of Science, and the University Court shall, after consultation with the Senatus Academicus, appoint such additional Examiners as they deem necessary to act along with them. Such additional Examiners shall be persons of recognised eminence in the subject of the thesis, or memoir, or work which is to be submitted for approval, and may be Professors or Lecturers in any Scottish University other than the University of Glasgow.

XIII. The thesis, memoir, or work submitted by a candidate for the degrees of Doctor of Science shall in each case be examined by the additional Examiner to be appointed by the University Court, as well as by the Examiners to be appointed by the Senatus under the provisions of Section XII of this Ordinance.

XIV. The result of the examination of the thesis, memoir, or work submitted by a candidate, as well as the result of the Examination prescribed under Section XI of this Ordinance, shall be reported to the Faculty of Science.

7. DEGREE OF DOCTOR OF PHILOSOPHY

The degree of Doctor of Philosophy was instituted by Ordinance LXXIV (Glasgow No. 21), which came into force in October, 1919. The relevant provisions of the Ordinance are as follows :

II. Research Students within the meaning of Ordinance No. 61 (General No. 23), who have prosecuted in the University of Glasgow, or in a College affiliated thereto, a course of special study or research in accordance with the provisions of that Ordinance, may offer themselves for the degree of Doctor of Philosophy, under the following conditions, namely—

(1) That they have obtained a degree in any Scottish University, or in another University or College specially recognised for the purpose of this Section by the University Court on the recommendation of the Senatus : provided always that a diploma or certificate recognised in like manner as equivalent to a degree may be accepted in place of a degree.

(2) That they have prosecuted a course of special study or research during a period of three academical years as Research Students in the University of Glasgow, or in a College affiliated thereto, and that they produce to the Senatus evidence of satisfactory progress in the special study or research undertaken by them during that period : provided always that the Senatus shall have power, in exceptional cases, to reduce the period to two academical years, and to permit a Research Student during part of the period to prosecute elsewhere his special study or research.

III. All candidates for the degree of Doctor of Philosophy shall present a thesis to be approved by the Senatus on the recommendation of a Special Committee appointed by the Senatus. The thesis shall embody the results of the candidate's special study or research, and shall be accompanied by a declaration signed by the candidate that it has been composed by himself. The Special Committee shall always include the Professor or other Head of a Department who has been appointed by the Senatus to supervise the candidate's work as a Research Student.

The University Court may, on the recommendation of the Senatus, appoint one or more additional Examiners to act along with the Special Committee in adjudicating on the merits of the thesis. The Senatus may, on the recommendation of the Special Committee, require the candidate to present himself for oral or other examination on the subject-matter of his thesis. A copy of the thesis, if approved, shall be deposited by the candidate in the University Library.

SUPPLEMENTARY REGULATIONS

1. Except in the case of members of the teaching staff of the University, the Senatus will not recognise Research Students as candidates for the degree of Ph.D. in the Faculties of Arts, Medicine, Science and Engineering unless they are able to devote the major part of the day during term-time to the object of their research.

2. In submitting a thesis a candidate must state, generally in the preface and specifically in the notes, the sources from which his information is derived, the extent to which he has availed himself of the work of others, and the portions of the thesis which he claims as original.

3. A candidate must submit two copies of the thesis and in addition two copies of a separate summary of the thesis. The separate summary (250–750 words) must be an adequate and informative abstract of the work, suitable for publication by the University. Both copies of the thesis, whether approved for the degree or not, become the property of the University.

8. DEGREE OF MASTER OF DENTAL SURGERY

The following regulations for the award of the degree of Master of Dental Surgery (M.D.S.) are contained in Ordinance CCXLI (Glasgow No. 56) which was approved by His Majesty in Council on 26th January, 1948.

XXII. Subject to the conditions hereinafter specified, the Degree of Master of Dental Surgery may be conferred on any candidate who has obtained the Degree of Bachelor of Dental Surgery of the University of Glasgow ; or, having obtained the Degrees of Bachelor of Medicine and Bachelor of Surgery, or the Degree of Bachelor of Science, of the University of Glasgow, holds in addition a Registrable Dental Qualification. The candidate must have produced certificates showing that, after having received his Registrable Dental Qualification, he has been engaged for at least one year in attending a Dental Hospital or the Dental Department of a General Hospital approved by the University Court, after consultation with the Senatus, or in the Naval, Military, or Public Health Services, or in practice in Dental Surgery.

XXIII. Each candidate for the Degree of Master of Dental Surgery shall be required to pass a clinical examination in Dental Surgery, and he may be admitted to that examination at such time, not sooner than one calendar year after he has received his Registrable Dental Qualification, as the Senatus may appoint for the purpose : provided always that in special circumstances the Senatus may, if they see fit, on the recommendation of the Board of Studies in Dentistry and the Faculty of Medicine, exempt a candidate from the whole or part of such examination.

XXIV. Each candidate for the Degree of Master of Dental Surgery shall submit for the approval of the Faculty of Medicine a Thesis on any branch of knowledge in the Second, Third, or Fourth divisions of the examination for the Degree of Bachelor of Dental Surgery, which he may have made a subject of study, excepting those subjects which are exclusively medical or surgical ; and the Thesis, accompanied by a declaration signed by the candidate that the work has been done and the Thesis composed by himself, shall be lodged with the Dean of the Faculty of Medicine on or before a date to be fixed by the Senatus. The Faculty may, if it sees fit, before approving the Thesis, require the candidate to present himself for oral or other examination on the subject-matter thereof.

9. DEGREE OF DOCTOR OF VETERINARY MEDICINE

By Ordinance CCLXI (Glasgow No. 66) which was approved by His Majesty in Council on 26th June, 1950, the Degree of Doctor of Veterinary Medicine (D.V.M.) may be conferred.

Regulations governing the award of the Degree have not yet been drawn up.

10. DEGREE OF MASTER OF VETERINARY SURGERY

By Ordinance CCLXI (Glasgow, No. 66) which was approved by His Majesty in Council on 26th June, 1950, the Degree of Master of Veterinary Surgery (M.V.S.) may be conferred.

Regulations governing the award of the Degree have not yet been drawn up.

RESEARCH STUDENTS

The following are the provisions of Ordinance No. 61 (General No. 23), by which the admission of Research Students is controlled :

I. It shall be in the power of the Senatus Academicus in each University, with the approval of the University Court, to make regulations under which graduates of Scottish Universities or of other Universities recognised by the University Court for the purposes of this Ordinance, or other persons who have given satisfactory proof of general education and of fitness to engage in some special study or research, may be admitted to prosecute such study or research in the University [or in a College affiliated thereto [1]].

II. It shall be the duty of the Senatus Academicus in each University :

(1) To receive and decide upon all applications for admission to prosecute special study or research ;

(2) To prepare a list of all persons so admitted (hereinafter referred to as Research Students) ;

(3) To make regulations for the supervision of their work ;

(4) To satisfy themselves from time to time that the Research Students are carrying on their work in the University in a satisfactory manner ;

(5) To suspend or exclude from any course any student whose conduct or progress is unsatisfactory.

III. Every applicant for admission must send in to the Senatus Academicus a written application stating any degree or other distinction which he has already obtained, the line of study or research which he

[1] Added by Ordinance XXXIX (Glasgow No. 12).

wishes to prosecute, and the probable period of its duration, together with evidence as to his character, capacity, and general qualifications.

IV. Any application for admission shall be in the first instance referred by the Senatus Academicus to the appropriate Faculty, or to a Committee appointed by the Senatus ; one member of the Committee shall always be a Professor or Lecturer within whose department the proposed line of study or research falls. No applicant shall be recommended by the Faculty or the Committee who has not satisfied them by examination or otherwise that he is qualified to prosecute the proposed line of study or research, and further :

(*a*) That his proposed line of study or research is a fit and proper one ;

(*b*) That he possesses a good general education ;

(*c*) That he is of good character ;

(*d*) That he proposes to prosecute his studies or research during a period to be approved by the Senatus Academicus.

The Faculty or the Committee shall make a report to the Senatus Academicus upon each application. It shall also be their duty, subject to the regulations of the Senatus Academicus, to provide for the supervision of the Research Student's work, and to report at least once a year to the Senatus as to his progress and conduct. The Senatus shall then determine whether he shall remain a Research Student.

V. Every Research Student shall be required to matriculate each year, paying the ordinary fee.

VI. Research Students shall have access to and the use of the University Laboratories and Museums, under such conditions as to payment and otherwise as the University Court, after consultation with the Senatus Academicus, may determine.

VII. The title of Research Fellow may be conferred by the Senatus Academicus, with the approval of the University Court, on Research Students who have shown special distinction. Such title shall not of itself confer any right to stipend, but it shall be in the power of the University Court to provide a stipend of such amount and for such period as it may think fit to any Research Fellow, under the powers of Section XI, sub-section 8, of Ordinances numbered 25 and 27, Section X, sub-section 8, of Ordinance numbered 26, and Section IV, sub-section 2, of Ordinance numbered 46.

VIII. (1) The Research Fellows shall be appointed as aforesaid after consideration of the report or reports submitted in terms of Section IV hereof.

(2) The title of Research Fellow may be conferred either at the commencement of the Research Student's course of study or research, or at any time during its progress, as the Senatus Academicus may determine.

(3) Research Fellows shall retain their title and stipend, if any, for the period during which they are engaged in special study or research in the University, and no longer.

(4) Research Students who have been appointed Research Fellows shall continue to be subject to the conditions above prescribed as to the supervision of their work, and the reports to be made thereon.

IX. Nothing herein contained shall prejudice the right of Research Students to such Fellowships, Scholarships, or prizes as may be open to them by Ordinance or Deed of Foundation.

X. The University Court may, subject to the provisions of Section XI, sub-section 8, of Ordinances numbered 25 and 27, Section X, sub-section 8, of Ordinance numbered 26, and Section IV, sub-section 2, of Ordinance numbered 46, provide such sums as it may think fit in aid of the expenses of special study or Research.

SUPPLEMENTARY REGULATIONS

1. Application for admission to the status of Research Student must be made to the Clerk of Senate on the prescribed form.

2. A Research Student may be required at the discretion of the Head of the Department to report his attendance to the supervisor of his research, or to the Clerk of Senate, at least once a week in term-time, except during periods when, with the permission of the Senatus, he is prosecuting his special study or research elsewhere than in the University or in a College affiliated thereto.

3. A Research Student may, with the sanction of his supervisor, attend classes in the University as a Private Student but he shall not be eligible for prizes in classes so attended and his attendance shall not qualify for graduation.

4. All papers arising out of work done in a department shall be submitted before publication to the Professor in charge of the department, and in all such papers, when they are published, a due recognition of the department shall be inserted.

CARNEGIE TRUST FOR THE UNIVERSITIES OF SCOTLAND

ENDOWMENT OF POST-GRADUATE STUDY AND RESEARCH

The regulations governing the award by the Trust of Scholarships, Fellowships and Grants in aid of research, are printed below. Nominations for Scholarships or Fellowships must be lodged not later than 15th March with the Secretary of the Trust, Merchants' Hall, Hanover Street, Edinburgh, from whom forms may be obtained. All Scholarships and Fellowships awarded in any year will normally date from 1st October.

A student who has not graduated but expects to take a degree at the summer or autumn graduation may be nominated for a Scholarship or apply for a Research Grant in the preceding March, but will not be eligible to receive either until he has graduated.

I. SCHOLARSHIPS

I. The Executive Committee are prepared to consider annually applications for Carnegie Scholarships in Science and Medicine and in History, Economics, English Literature and Modern Languages.

II. The number of Scholarships for annual award is limited. Applicants for Scholarships in Science, more especially where the proposed research is in Physics or Chemistry, are requested to note that, in consideration that maintenance allowances for training in research are available from public funds administered by the Department of Scientific and Industrial Research, the Executive Committee must reserve to themselves the right to give preferential treatment to applicants ineligible to receive allowances from the above-named source.

III. Scholarships will be of the annual value of £300. They will be tenable for one year in the first instance and, subject to satisfactory reports, be renewable for a second year. They may, in exceptional circumstances, be extended to a third year. Unless with the express consent of the Executive Committee the holder will be expected to devote his whole time to the purpose for which the Scholarship is awarded.[1]

IV. Applicants for Scholarships must be graduates of a Scottish University.[2] The standard required for applicants holding Degrees in the Faculties of Arts and Science is First Class Honours.

V. Applicants for Scholarships must be nominated by a Professor, Reader or Lecturer in a Scottish University, or by a Teacher in Scotland recognised for purposes of graduation by a Scottish University. In assessing the suitability of applicants for Carnegie Scholarships nominators will be expected to take into account the awards obtainable for research from such bodies as the Department of Scientific and Industrial Research, the Medical Research Council and the Agricultural Research Council.

[1] In the event that applicants are not considered sufficiently advanced to warrant the award of the full Scholarship of £300, the Executive Committee may award a Minor Scholarship of £200 for one year.

[2] *Note.*—For the time being the Executive Committee will be prepared to consider applications from students who expect to graduate within a short time of making their application.

VI. The Executive Committee may, at their discretion, supplement Bursaries. Scholarships and Fellowships gained by graduates of the Scottish Universities. Applicants for such supplements must furnish a certificate from the Secretary or Registrar of their University showing the title, value and length of tenure of the Bursary, Scholarship or Fellowship gained, and will be required to conform to the regulations framed by the Executive Committee for holders of the full Carnegie Scholarships.

VII. Scholars will be required to work under a supervisor who need not be the Professor, Reader or Lecturer nominating them. Quarterly reports will be expected from the Scholars and their supervisors. Payments of the Scholarships will be made quarterly, the second and subsequent quarterly payments being dependent on the receipt of satisfactory reports.

2. SENIOR SCHOLARSHIPS

I. The Executive Committee are prepared to consider annually applications for a restricted number of Senior Scholarships in Science and Medicine, and in History, Economics, English Literature and Modern Languages.

II. The Senior Scholarships will be of the annual value of £350 together with a sum not exceeding £100 for fees, books and any necessary travelling expenses. They will be tenable for one year in the first instance but may be renewed for a second and, in exceptional circumstances, a third year. Payment will be made in quarterly instalments.

III. Applicants must be graduates of a Scottish University and be able to adduce proof of successful research for a period of at least three years prior to the application. Applications are not confined to those who have previously held Carnegie Scholarships of £300 per annum.

IV. Applicants must be nominated by a University Professor, Reader or Lecturer in a Scottish University, and must submit the names of two authorities, other than the nominator, to whom reference may be made if deemed necessary. Successful applicants will work under the general direction of a supervisor from whom, twice annually, a certificate will be required attesting satisfactory progress. The Senior Scholar will himself furnish reports twice annually on the progress of his work. He will be required to give his full time to the purpose for which the Senior Scholarship is awarded unless with the express sanction of the Committee.

3. FELLOWSHIPS

I. The Executive Committee are prepared to consider applications for Carnegie Fellowships in Science and Medicine and in History, Economics, English Literature and Modern Languages.

II. The applicant must be a graduate of a Scottish University or a member of the staff of one of the Universities or Colleges in Scotland receiving grants from the Trust.

III. The number of Fellowships will be limited. Their annual value will not, normally, exceed £800 per annum together with a sum not exceeding £50 for expenses in instances in which such grant is considered necessary. The period of tenure will, normally, be one year but special periods may be arranged.

IV. Nominations of persons holding posts in one or other of the Scottish Universities will be made by the appropriate University Authority ; nominations of graduates not so employed will be made by a Professor, Reader or Lecturer in a Scottish University. Arrangements will be made where necessary to maintain the employer's contribution to the Federated Superannuation System for Universities or similar scheme.

V. The Carnegie Fellows will undertake to give full time to the programme of research in respect of which the applications are made, but may, at the discretion of the Committee, engage in a limited amount of higher teaching or instruction associated with the special nature of their research work. They will come under an obligation to furnish a report once in each year on the progress of their work.

SPECIAL AWARDS

The Executive Committee are prepared to consider applications for special awards to be made for the execution of particular research projects which may not fall within the scope of the foregoing Regulations.

4. GRANTS IN AID OF RESEARCH

I. An applicant for a Research Grant must be a Scottish University Graduate resident in Scotland, or an actual member of the staff of one of the Universities or Colleges in Scotland receiving Grants from the Trust.

II. Applications must be made on a form which can be had from the Secretary of the Trust.

III. Applicants proposing to engage on research must furnish information on the following points at the time of making a first application.

(1) Their experience in research, with copies of or references to any published papers ; or, if they have no papers to offer, with references to two or more authorities who are acquainted with their qualifications for research.

(2) The nature of the research in which they desire to engage, and the results expected to follow therefrom.

(3) A statement of special requirements for the proposed research, with a detailed estimate of the cost.

(4) Whether they have received, or are receiving, any grant from any other source for the same object ; and if so, what results have already ensued from their investigations.

NOTE.—*Second or subsequent applications need not be accompanied by copies of published papers.*

IV. By accepting a Grant applicants come under an obligation to pursue the programme of research which has been approved, and to send to the Executive Committee a report containing (*a*) a brief statement (not necessarily for publication) showing the results arrived at, or the stage which the inquiry has reached ; (*b*) a statement of the expenditure incurred ; and (*c*) copies of or references to any papers in which results of the research have been printed.

The Executive Committee expect that in every case the results of the research will be published in some form. Copies of the published records of all work carried out with the aid of a Grant must be forwarded to the Offices of the Trust without delay.

V. A Research Grant is not intended (1) to take the place of such provision as should be made by the University Courts out of the Grants for Permanent Equipment under the Scheme of " Grants for five years to the four Universities of Scotland," or (2) to provide minor apparatus or research materials or instruments which should form part of the equipment of a Laboratory appropriate to the investigation.

VI. Grants are not applicable to the payment of salaries, wages, or honoraria, except in so far as they may be assigned for a specific purpose, as, for example, the cost of preparing necessary illustrations as specified in the application and approved by the Executive Committee. Such illustrations may include drawings, photographs, or maps.

VII. Applicants for guarantees against loss on the publication of books must, at the time of application, submit such MSS. as will enable the Trust's advisers to arrive at a recommendation in regard to the application.

VIII. Grants in aid of illustration to an amount not exceeding three-fourths of the estimated cost may be applied towards the preparation of process blocks or other means of reproduction, and of the actual printing of the illustration, including any special paper necessary for the purpose. Grants are not applicable to the cost of printing and publishing the letterpress of the publication unless, in exceptional cases, special tabular matter is required. Applicants for such grants in aid must, at the time of application, submit either an off-print of the publication or such MSS. as will enable the Trust's advisers to arrive at a recommendation in regard to the application.

Note :—Certain grants in aid of the publication of papers on the *Transactions*, *Proceedings* or *Journals* of learned societies in Scotland are now paid direct to the society and not to the individual contributor.

IX. Applications for Grants in aid of Laboratory research, or of the adequate publication of its results, must be made by the individual workers concerned, and Grants made for specific purposes to one worker cannot be utilised by another (whether in the same Laboratory or not) without the express consent of the Executive Committee.

X. An application for a Grant to be used by two or more collaborators in the same research must be signed by each ; but they shall appoint one of their number who shall be responsible for furnishing the report, for receiving and disbursing the money, and in general, for the conduct of the research.

XI. Instruments of permanent value purchased by means of the Grant shall remain the property of the Trust, but at the conclusion of the research, or at such other time as the Executive Committee may determine, they shall be placed under the care and at the disposal of the institution in which the research has been conducted, provided that the Executive Committee may, if they see fit, request their return.

5. TRAVEL AND MAINTENANCE ABROAD ON RESEARCH

I. In extension of its existing Research Scheme, the Trust proposes, as a tentative measure in the first instance, to invite applications from members of the staff of a Scottish University who wish to pursue research abroad for a limited period.

II. The essential object of the scheme is to give an opportunity to advanced investigators in Science and Medicine and in History, Economics, English Literature and Language and Literature other than English to pursue their particular research work abroad for such periods as may seem advantageous.

III. Normally the period for which application is made shall be not less than three or more than twelve calendar months.

IV. The amount of the award shall be such as may be found requisite having regard to the expense to be incurred in travel and additional maintenance, but shall, in no case, exceed £600.

V. The number of awards to be made in any one academic year will depend upon the nature and extent of the applications received but should not, normally, exceed 10, not more than 4 of which will be tenable in the United States of America.

VI. Members of the staff of a Scottish University shall be nominated by the Principal of the University. Full details of the proposed investigation together with the estimated cost thereof must be forwarded with the nomination.

VII. Successful applicants will be responsible for making all arrangements for travel and for residence abroad. The Trust will supply what information is required to enable the applicants' bankers to make the necessary representations to the Bank of England but cannot undertake to arrange details of the journey and residence.

VIII. Upon their return applicants will be required to furnish a report to the Trust giving, *inter alia*, such details as will be useful to the Executive Committee in their future consideration of this scheme as a whole.

IX. Special forms of application can be had on request from the Secretary and Treasurer at Merchants' Hall, 22 Hanover Street, Edinburgh, and must be returned to him not later than 1st February in each academic year.

X. Applicants must be not less than 30 years of age at the time of application.