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Handling Mosquitoes for Experimental Purposes under South African Conditions.

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WHILE engaged in work on the transmission of horsesickness and bluetongue we commenced our experiments with mosquitoes and more especially with different *Aëdes* species, which, for reasons stated in the previous paper had to be regarded as the most probable transmitters. First of all suitable methods had to be evolved for rearing these insects, feeding them on the experimental animals and keeping them alive for a sufficient period under South African conditions. That there are typical South African conditions is shown at a glance from the following experiences:

When putting mosquitos contained in a small wire cage covered with mosquito netting, on stabled horses in Europe for one night, on an average, perhaps 70 per cent. will have fed and only a few, if any, will have died by the next morning. Repeating the same experiment in South Africa during a dry summer night, one will generally find, that the next morning 80-90 per cent. of the mosquitoes are dead and only a few specimens engorged. The adverse climatic conditions are responsible for this and this has proved to be the most important obstacle in any mosquito work out here.

We had to work out our methods from practically the beginning, as there were no former experiences on these lines of any real value. In fact, every earlier attempt to work with mosquitoes in connection with horsesickness had to be given up on account of technical difficulties.

We started our experiments with practically no equipment at all and everything had to be specially designed and constructed. The fact that the methods we used had to be altered from time to time during the course of the work to fulfil the requirements of the changing climatic conditions was unavoidable.

We feel sure that the methods finally adopted are not absolutely ideal and further improvements can certainly still be made, but they enabled us, at any rate, to carry out the main transmission experiments. This work being limited, as it was, to one season only, had to be tentative in nature as it was impossible to predict the influence of the climatic conditions of the whole season upon the mosquitoes under experiments conditions, on account of our being able to devote only part of our time to these problems. We were, therefore, not able to investigate thoroughly the reasons for every failure or unsatisfactory result. Apart from this, on account of the expense involved, we had to give some of the elaborate apparatus, which took months to make, a thorough trial before suggesting further alterations.

A somewhat complete description of our technical observations and results seems to be indicated as a guide for further work on these problems.

We wish here to express our thanks to Mr. Appleton, who most ably assisted us in constructing the cages, stables and other devices used during this work.

1. CATCHING OF ADULT AND LARVAL MOSQUITOES.

The mosquitoes used in our experiments were collected as larvae and pupae or as adults.

The *larvae* and *pupae* were caught in the usual manner by means of a strong piece of wire bent at one end in the form of a loop about 4-5 inches long by 2-3 inches broad, which was covered with a double layer of ordinary mosquito netting. As a rule, only nearly fullgrown or fullgrown larvae and pupae were collected, as these required little attention in the laboratory.

The larvae and pupae were kept, until the hatching of the adults took place, in large glass jars, by preference, in water from their breeding places, as this supplied them with the necessary food. Extra food was usually not necessary, but in cases where a supply of food seemed to be wanted, powdered rice was found to be suitable as it was readily taken up by most of the larvae and only seldom caused putrefaction of the water. Overstocking of the jars must be avoided, as this, apart from giving rise to a shortage of food, leads to retardation of development or a high mortality, due to the amount of excreta passed into the water.

Care must be taken that all carnivorous insect larvae are eliminated from the jars. Beetle larvae may cause serious losses, but the most important, in our experience, were brought about by the larvae of two mosquitoes, *Mucidus scatophagoides* and *Lutzia tigripes*, which are often associated with the larvae of *Aëdes caballus*, *A. lineatopennis* and *A. hirsutus* in their breeding places, and which may devour a surprisingly large number of these larvae. In one experiment in the laboratory one larva of *L. tigripes* devoured, during the 10 days of its development, 43 larvae of *A. hirsutus*, viz., 16 halfgrown larvae during the first 4 days and 27 fullgrown during the last 6 days. The larvae of *Mucidus*, which are much larger than those of *Lutzia*, will require even more larvae.

The jars containing the larvae were covered with mosquito netting to prevent the newly hatched adults from escaping. For catching the adults the jars were placed in front of a window, the light attracting the insects to one side, part of the netting was lifted up and the mosquitoes caught in small tubes.

The mosquitoes had to be collected within a day of their hatching out as, especially when large numbers were hatching out together, they were liable to be drowned. When many mosquitoes had to be handled at the same time, a number of jars containing the larvae were sometimes placed together without cover in a sufficiently large cage. The number of drowned specimens was thereby considerably reduced and the catching of the mosquitoes could be extended over a number of days.

For catching and handling the mosquitoes, small test tubes, 6 inches long by ⅜ inch wide were found to be the most suitable. They are just large enough to allow the mosquito to enter, and small enough to prevent the insects from escaping easily. Large test tubes are not suitable on account of the ease with which the insects escape.

For catching *adults in the field* large hand nets were used with which the grass of the breeding grounds was swept. Catching the mosquitoes by means of tubes between the blades of grass was very difficult. Of an afternoon, when no strong wind was blowing, we found that the best method was often to wait until the mosquitoes, disturbed by our walking through the breeding grounds, settled down on ourselves or the boys. They were then easy to catch by means of tubes. This had the further advantage that only undamaged specimens, ready to feed, were caught. In this manner we obtained the adults of *Aëdes caballus* and *A. lineatopennis* in large numbers and besides them some *A. durbanensis* and *A. nigeriensis*. The resting places of the other mosquitoes were not located. It may be mentioned that walking through the country and sweeping the grass here and there, is very seldom of any use as, at least in the case of *A. caballus* and *A. lineatopennis*, the resting and shelter places are very restricted to certain spots, as has been stated in the review of the results of our mosquito survey. When walking through a typical resting place, the mosquitoes are disturbed and fly up in large numbers, when they are easily noticed.

The greatest difficulty encountered was the collection of sufficient material this season due to the shortage of rain and consequent scarcity of natural breeding places. Anophelines were very rare throughout the season and no way of assisting their breeding by artificial means could be found. The number of *Aëdes* occurring naturally was also not very large. However, we were able to make provision for some species at any rate, especially *A. caballus*, *A. lineatopennis* and *A. hirsutus*, by flooding known breeding grounds either with tap water or with irrigation water. The results were, in most cases, very satisfactory indeed, but owing to the general shortage of water we were unable to resort to this method as often as we wished. The methods employed and the difficulties in getting the water to the right spots have already been discussed in the preceding paper.

2. METHODS FOR KEEPING MOSQUITOES ALIVE IN THE LABORATORY.

The mosquitoes, which had hatched out from larvae, been collected in the field or had been caught as adults, had to be kept in the laboratory for some days to start with before they could be fed on an infected animal, and thereafter, until they were again put on to susceptible animals.

Freshly emerged adults do not feed directly. Their chitin must first harden thoroughly. From the second to third day after their hatching out they can be used for experiments. They had often, however, to be kept longer before use as only after certain intervals could animals be infected to feed them on.

Horsesickness is most likely not transmitted mechanically by mosquitoes, supposing these insects are the natural carriers. In all probability some sort of development, or at least multiplication of the virus has to take place in the mosquitoes before they become infective. In analogy with other diseases, yellow fever of man for instance, this incubation period in the insects is stated to last 10-15 days. It was therefore necessary to keep the mosquitoes alive for at least this period, preferably longer.

As is the case with most insects, *temperature, humidity* and *food* are the essential factors on which the mosquitoes depend. We knew that former experiments at Onderstepoort with mosquitoes had failed on account of their dying rapidly in captivity, and we had therefore to go somewhat deeper into these problems.

The mosquitoes require a certain *minimum temperature* for their existence and ordinary room temperature would have been sufficient during the summer months. However, the development of the virus would very probably be influenced by the temperature to a considerable extent. A certain (yet unknown) minimum will be necessary for the development to take place at all, whereas with increases in temperature the incubation period in the mosquitoes will be proportionately shortened. A high temperature was the most suitable for the virus, whereas at relatively low temperatures the mortality amongst the mosquitoes was the smallest. The optimum had to be regarded as the highest temperature just not detrimental to the mosquitoes. This optimum seems to lie in the neighbourhood of 26-28° C. We had at our disposal a warm room, not specially built for entomological purposes however. It was electrically heated but the heating had to be regulated by hand so that a constant temperature was therefore not obtainable. During the winter and early summer months the heating was on day and night, giving an average temperature of 24-26° C. During the later summer months the room was only heated during daytime as no control could be exercised at night and sometimes very high temperatures were recorded. As far as possible the temperature was maintained at an average of 26° as, on account of the insufficient means of control, an average of 28° was regarded as too risky. From time to time the temperature varied between 20 and 30° C.

To provide the mosquitoes with the necessary degree of humidity they were stored, in the first series of experiments, in ordinary jam jars, the lids of which were replaced by mosquito netting. Ten specimens were put into each jar, which was also provided with some strips of filter paper. The jars were kept on wet cotton wool in slightly larger jars provided with metal lids. These lids had holes in them to allow of the escape of excess water vapour, as absolutely saturated air is unfavourable for the mosquitoes. The mosquitoes were fed on sugar water, a small piece of cotton wool soaked in a 10 per cent. solution being placed on the netting covering the jars. The jars had to be changed from time to time when they became soiled by the excretions of the mosquitoes or when deaths occurred. This method of keeping mosquitoes worked quite satisfactorily, especially when the mosquitoes were needed for injections and when only a small number had to be handled. Difficulties, however, were encountered with large numbers and when mosquitoes were being prepared for feeding by this method.

Some mosquitoes take up a very large amount of sugar water especially when confined in a small space, and many again will not feed very well. The best way of preparing mosquitoes for feeding is to put them on plain water only for a day or two and give them no fluid at all during the last 12 or 24 hours, keeping them at the same time at high temperatures so as to reduce the fat bodies if present. The difficulty experienced with mosquitoes kept in jars was that some would take up large amounts of sugar water, whereas others remained quite empty, and on starving them for some time, some would die before others would be ready for feeding.

Aëdes caballus is an exception to this rule as it will feed readily even when the abdomen is greatly distended with sugar water.

Later on large wooden cages, 36 inches long, 22 inches broad and 24 inches high, which were available at the laboratory, were used. These cages consisted of a framework of wood covered on three sides by mosquito netting, the one end being so constructed as to form a hinged door to facilitate cleaning or the placing of large objects inside the cage. The remaining side contained two glass panes, one on each side, to ensure good visibility from outside, and the centre panel, of mosquito netting, was provided with a thick wire ring, 4 inches in diameter, held in place by means of metal supports, to which was attached a sleeve of mosquito netting. Through this sleeve mosquitoes could be introduced or caught out of the cage with little chance of their escaping. The top and bottom of the cage were constructed of wood, overlapping the cage by about 1 inch. A sheet of galvanized iron, bent down at the sides, covered the top and a piece of hessian, of the same size as the galvanized iron top and having four flaps corresponding in size to the four sides of the cage, completely covered the cage. To the lower end of each hessian flap a piece of $\frac{1}{4}$ inch rod iron was sewn and these, when wedged in under nails driven horizontally into the edges of the floor of the cage, served to hold the hessian taut. Waste water was led away in a metal gutter attached to the bottom edge of the cage just below the lower ends of the hessian flaps.

To ensure a sufficiently high degree of humidity inside the cage the hessian cover was kept wet by running water on to the top of the cage. Water was supplied either direct from the mains or from an ordinary four gallon petrol tin standing on bricks on the roof of the cage and fitted on the underside with a gas tap. Once the hessian was wet a surprisingly small amount of water was needed to keep it so, and the contents of a petrol tin was more than enough to last out overnight.

Big balls of cotton wool, tied up in mosquito netting and soaked in a 10 per cent. solution of sugar water, were suspended from the roofs and supplied the mosquitoes with food. These balls had to be changed every second day on account of the growth of moulds, and were sterilized before being used again.

The results obtained by this method were quite satisfactory. The mortality amongst the mosquitoes was not excessively high and without special starving a large percentage was ready to feed, as could be noticed when introducing the hand into a cage. The only drawback was that different species of mosquitoes or different groups of the same species could not be separated. We finally adopted smaller specially constructed cages, consisting of a wooden framework, 21 inches long, 10 inches broad and 10 inches high, which could be

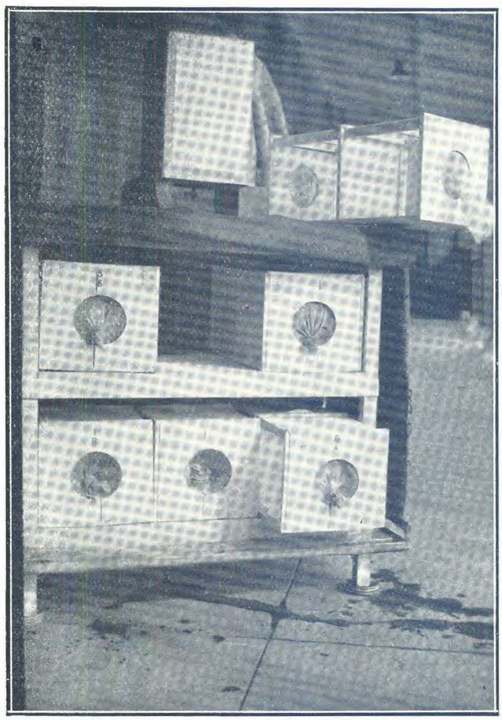

FIG. 1.

separated into two compartments, if necessary, by a sliding sheet of galvanized iron in the centre. They were covered on the two long sides and top with mosquito netting, the bottom and the two ends being made of wood. Each end contained a hole, large enough to admit the hand, and was fitted with a sleeve of netting. A framework, capable of holding 6 of these cages in two tiers of three, was constructed, and in order to ensure the desired degree of humidity inside the cages, the arrangement described above was used, viz., a metal top covered with hessian, having flaps hanging down on all sides, and a gutter for conducting away the waste water (c.f. fig. 1).

The results were practically as good as with the other methods and may be regarded as satisfactory. They were certainly not yet optimal and in the case of certain species better than with others. We do not yet know the reasons for some of the failures, but differences from the optimum humidity are in all probability the main factors. We had, however, not sufficient time and equipment at our disposal to go deeper into these problems.

With *Aëdes hirsutus* the results were very satisfactory indeed, only an insignificant mortality occurring. Only females, reared from larvae, were used. In one experiment, e.g. in March, 1932, 311 mosquitoes were fed on an infected horse, and after a period of 7 days 100 specimens were killed and injected into a horse, the others being used for feeding experiments. Of the remaining 211 specimens, 157 were alive 15 days after the initial feeding, 107 after 20-21 days, and 46 after 29-30 days. The conditions obtained in the cages were thus apparently quite favourable for *A. hirsutus*.

With *Aëdes lineatopennis* the results were also quite satisfactory. In one experiment carried out in March as well, for which mosquitoes reared from larvae and also specimens caught as adults were used, 151 specimens were fed on an infected horse. After a period of 19-21 days 71, or nearly 50 per cent., were alive, after 25-27 days 42, more than a month (33-35 days) after the initial feed, 38 specimens, and after an interval of as long as 60-62 days, 11 mosquitoes were still present. This result may certainly be regarded as good. In another experiment during the same month, 49 specimens out of 109 mosquitoes, or nearly 50 per cent., were still alive after a period of 16-18 days. In some of the other experiments the mortality was slightly higher. Even oviposition did not seem to affect this species very much, whereas in the case of *A. caballus*, the mortality, after the deposition of eggs, was considerable. On the whole we may say, that the conditions obtained in the cages were not unfavourable for *A. lineatopennis*.

Aëdes caballus was the most difficult species to keep alive for any length of time in captivity. When kept in the same manner as the other species the mortality was much higher. We endeavoured to still further raise the humidity by putting sheets of wet filter paper on the floors of the cages, but this did not help to decrease the mortality. In fact, the best results were obtained in our early experiments, when 10 specimens were stored in each of a number of ordinary jam jars. Adults caught in the field were more liable to die than those bred out from larvae in the laboratory, especially was this the case after oviposition, when the mortality was exceedingly high. As an illustration of the results obtained with this species, some experiments may be mentioned. In one group of experiments, 816 specimens were fed on an infected horse. Two weeks later only 100 specimens were left, and after a further week, not more than 10. In another set of experiments with a different strain of virus, out of 132 specimens used for injection into a horse, 68 mosquitoes, or about 50 per cent., were alive one week after the first feeding. Out of more than 900 specimens, which were used in feeding experiments, only about 10 per cent. survived 15-21 days. The highest mortality occurred as usual at the end of the first week after the initial feeding, when eggs were deposited. Although on this occasion most of the groups consisted of mosquitoes reared from larvae, eggs were frequently laid. Females and males had hatched out together in the large cages, and before use had remained there together for some time, during which copulation must have taken place. The results were thus not ideal, but a certain number of specimens could be kept alive for the time required in our experiments. Furthermore the relatively high mortality was not too serious as large numbers of this species could be obtained by artificially flooding their breeding grounds.

Aëdes dentatus was more easily kept alive under laboratory conditions, however, not to the same extent as *A. hirsutus*. Out of 61 specimens, e.g., fed on an infected horse, 22 were still alive 25-27 days later, and 6 after 35-37 days. In another experiment, 129 mosquitoes engorged themselves on a horse and of these 63, or nearly 50 per cent., were still alive at the end of 14-21 days.

Lastly, *Aëdes vittatus* was not difficult to keep alive in captivity, Our supply of this species was rather limited however.

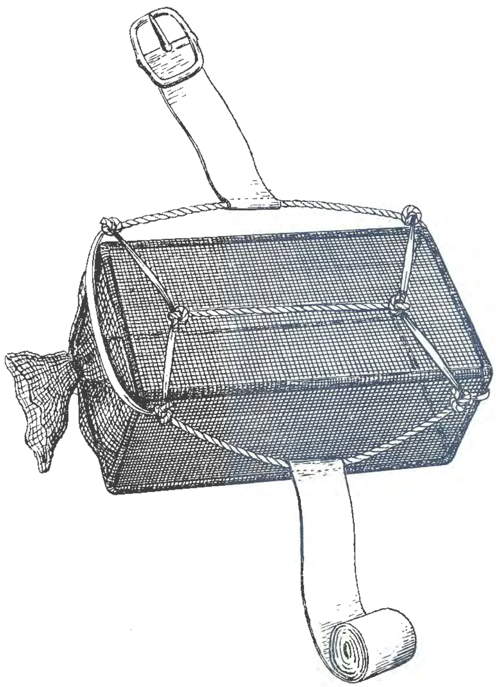

Fig. 2.

3. METHODS OF FEEDING MOSQUITOES ON HORSES.

(*a*) Cages for Feeding Mosquitoes.

For feeding the mosquitoes on horses and sheep small cages, covered with mosquito netting, were used. These cages, containing the insects, were attached to the skin of the animals and were left there overnight. Cages of this sort had previously been used by one of us with good results in Europe for similar mosquito work (Nieschulz and Bos, 1931).

The cages were 5 inches long, 3 inches wide and $2\frac{1}{2}$ inches deep, and consisted of a framework of strong wire, covered with a single layer of mosquito netting at the bottom and a double layer on the other sides (c.f. fig 2). A double layer of the ordinary mesh netting was necessary, otherwise small specimens could escape, especially at the edges. The netting projected at one of the small ends of the cage to form a sleeve 3-4 inches long through which the mosquitoes could be introduced and caught out later. The sleeve could be securely closed by tying it with tape. The measurements of the cages appeared to be optimal for our purposes. Larger cages were unsuitable as they did not fit flat on to the skin of the horse and left a space between the skin and the netting at the bottom of the cage which interfered with the feeding of the mosquitoes. Smaller cages would not have been capable of holding a sufficient number of mosquitoes. Our cages could quite easily accommodate 100-150 specimens without danger of their disturbing one another. For collecting the fed specimens from the cages, small test tubes, 6 inches long and $\frac{3}{8}$-inch wide, were found to be most satisfactory on account of the small bore preventing the mosquitoes from leaving them easily and therefore as a rule remaining in them until the tube was removed from the cage and the opening plugged.

(*b*) Feeding Mosquitoes set Free in a Tent.

In a few experiments in February, 1932, the cages were not used. An infected horse was put into a box surrounded by a mosquito-proof tent standing in the veld near the laboratory, and a large number of newly hatched mosquitoes were set free in this tent. Quite a number of the insects engorged themselves, at any rate in two of the experiments, but in comparison with the numbers involved the results were by far not so good as with the cages. In the other experiment the result was not at all satisfactory.

(*c*) Arrangements for Fixing the Cages on the Horses.

The mosquitoes were fed on the backs of the horses, a patch of hair corresponding to the size of the cage being clipped short. Shaving was not necessary.

To start with we attached the cages to the horse by means of strips of ordinary plaster stuck to shaven areas around the clipped patch of skin. To the bottom edges of the cages a piece of calico about 1 inch broad was sewn, to which the plaster was fastened. The horse as put into a box which somewhat restricted its movements and each of the cages, attached to its back, was fastened to the ceiling of the stable by means of a piece of string which was sufficiently slack not to dislodge the cage should the horse move. If the animal fell down, e.g., when dying, the cages would be lifted off its back, hang on the strings, and thereby escape damage. This method worked quite satisfactorily and often over 90 per cent. of the mosquitoes were found engorged the next morning. However, difficulties were encountered when the horse was restless and reacted to the bites of the mosquitoes by twitching its skin. The insects were disturbed, which resulted in only a small number of specimens engorging themselves, or, it became loose before the mosquitoes could feed at all.

In trying to find a more suitable method of attaching the cages properly to the skin of the horse two strong rubber bands were put over the cage in the manner illustrated in fig. 2 to which two strips of strong linen (or leather straps) were attached. The one strip, which was long, went over the back and around the animal like a girth and was fastened to a short one on the other side of the cage. The rubber bands allowed for the normal movement of the animal's chest at the same time holding the cage in position and, in most cases at any rate, the results were quite satisfactory. This method was certainly an improvement but was still far from ideal.

Finally we had a special *saddle* constructed. It consisted of two galvanized sheet iron " saddle flaps " hinged on to a section of the same material, as shown in fig. 3. The saddle was lined by thick felt rivetted into position along the edges and each flap contained rectangular openings large enough for the feeding cages to fit easily. This saddle was held in position by means of an ordinary girth. The felt protected the animal from injury by the metal and at the same time tended to immobilize the skin when the horse was irritated by the bites of the mosquitoes. The cages containing the mosquitoes, after

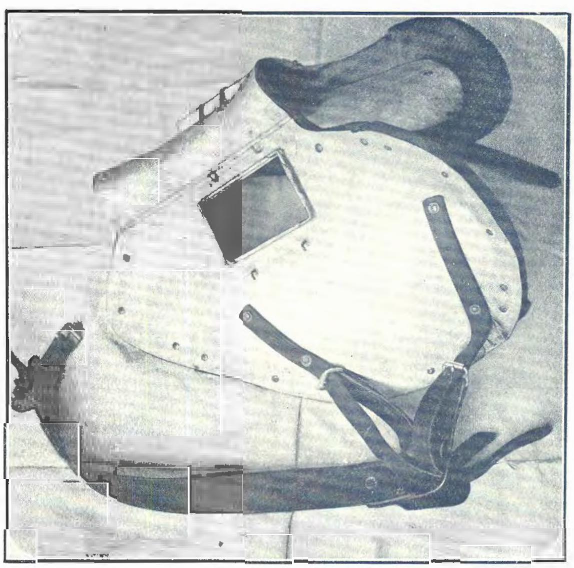

FIG. 3.

being inserted into the rectangular holes of the saddle, were pressed down on to the skin by means of rubber bands which were fixed to hooks of which a pair was soldered on to the saddle flap above and below each opening. The pressure upon the cage could be varied by the number of rubber bands used and could be increased to such an extent that no movement could take place. In fact one had to be careful that the pressure was not too great as the cages often left the imprints of their frames on the skin, or even pressure necrosis resulted.

No special precautions were taken in cases where the horse fell or lay down, and this occasionally happened when the animal died overnight. The result, however, was that only the framework of some of the cages was bent, as only a portion of it projected outside, and the remainder was covered by the saddle which protected it to a certain extent. The mosquito netting covering the cages was never damaged and thus no mosquitoes could escape.

This method was very satisfactory in every case and may be regarded as the method of choice when working with horses. The mosquitoes are not disturbed at all, a large percentage engorge themselves, and the cages are very easily put into position on the horse and removed again.

(*d*) Feeding Mosquitoes in Ordinary Stables.

For the first experiments with horses, in September, 1931, only *Culex theileri* was available. The mosquitoes were fed in the small cages attached to the skin of the horse by strips of plaster. The horse itself was placed in a strong wooden box, just large enough to allow of some little movement, but preventing the animal from lying down. The box stood in an ordinary stable and a wide mosquito net was hung over the horse and box. Later on the use of this net was discontinued as it was found that no mosquitoes could escape from the cages. The experiments with *C. theileri* were concluded in October and the results obtained were satisfactory.

In November experiments with *Aëdes* species were commenced. The feeding results were not bad in November and the first part of December, but in some cases the percentage of engorged specimens was markedly less than was the case in the experiments with *Culex*, and a certain mortality, which occurred during the time the mosquitoes were on the horses, was also observed. This mortality later became very considerable, making experimental work almost impossible, as at times during January it rose to 90 per cent. The technique having remained the same, the changing climatic conditions alone could have been responsible.

(*e*) Influence of the Climatic Conditions on the Feeding Results.

In February, 1932, some experiments with mosquitoes set free in a fly proof tent were conducted. In the first experiment carried out on 1st February, during a wet night, the feeding results were good. The second experiment on 12th February was most unsatisfactory. Well over 1,000 specimens were liberated in this tent but only 95 engorged themselves on the horse and a very large number died without taking any food. It had been a dry day followed by a dry night and this failure strengthened our viewpoint that the prevailing low degree of humidity was responsible for the poor result. In order to verify this opinion we erected a similar tent near one of the buildings of the laboratory and fixed a hose above it in such a way that water could be run on to the roof and down the sides. The distribution was far from ideal, however, on account of the somewhat crude construction and, whereas a strong stream flowed down the one side, the other remained practically dry (fig. 4). The ground around the tent became thoroughly soaked and the splash of the falling water combined to create a fairly humid atmosphere. The mosquitoes were again set free in the tent on 17th February and, notwithstanding the dry conditions which had prevailed during the day and night, the result was much better, almost 350 specimens engorging themselves on the horse. This constituted the final proof, so far as we were concerned, viz., that in order to obtain good feeding results, at any rate during the summer months, a humid atmosphere was an absolute essential.

(*f*) The Special Stable for Mosquito Feeding.

During the experiments which followed we used the same tent even when feeding mosquitoes in the small cages, and consistently good results, which were quite independent of outside weather conditions, were obtained. The tent was not an ideal place in which to work, however, and finally a special stable was designed and constructed, as shown in fig. 5. It consisted of a wooden framework 11 feet long, 8 feet wide and 7 feet 6 inches high. The roof was flat and consisted of a wooden framework covered by sheets of galvanized iron soldered together so as to render it water-tight. Mosquito netting covered the four sides, the lower edges of which were protected by flooring boards nailed to the uprights. The front side contained two doors, a large almost central one, measuring 6 feet 11 inches by 3 feet, through which the horse could be led in and removed, and a small door at one side, 33 by 34 inches, which gave us access to the stable. This small door was purposely made as small as possible so as to

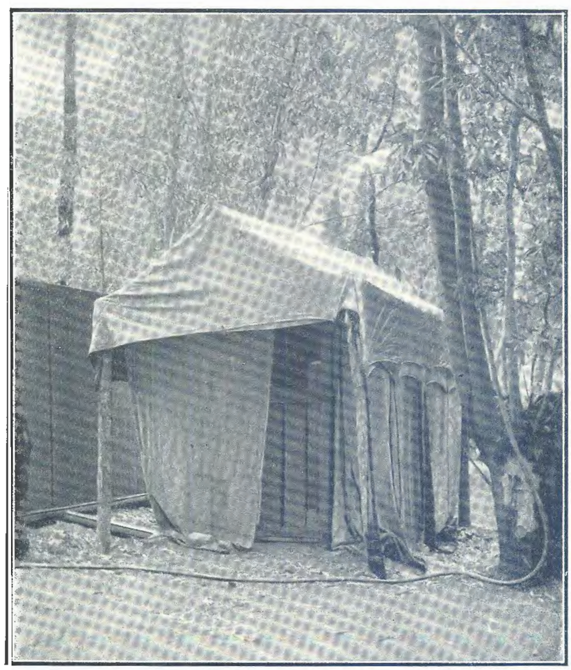

Fig. 4.

minimize the chances of mosquitoes which had perhaps got out of one or other of the small cages, escaping. The whole stable was absolutely mosquito-proof and insects other than those used in the experiments were thus prevented from coming into contact with the horse at night. The importance of these precautions, especially in the case of susceptible horses, is self evident.

The whole stable, top and all four sides, was covered with hessian which could not come into contact with the mosquito netting on account of the roof overlapping the rest of the structure by 6 to 8 inches. In front the hessian was divided and hung down in the form

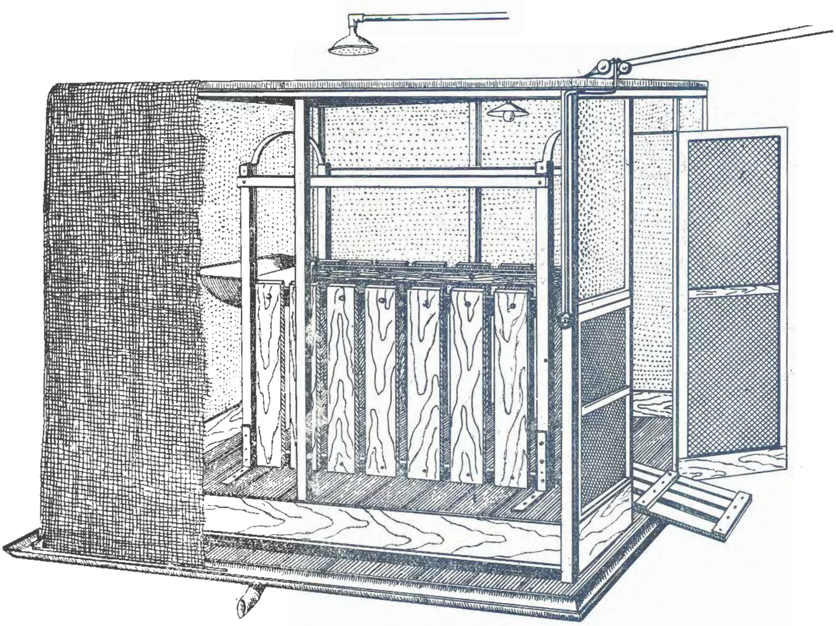

Fig. 5.

of curtains which could be lifted up so as to expose one or other of the doors. Along the lower edges of the hessian pieces of ½-inch rod iron were sewn which served to hold the hessian straight. Around the bottom of the stable galvanized guttering was attached into which the hessian hung, it being held taut by stout nails driven horizontally into the floor of the stable through the inner side of the guttering and under which the iron rods were forced.

Above the centre of the stable an ordinary bathroom shower was fixed but this was later replaced by a small garden spray, which spread the water more evenly and gave better results. On opening the water tap the hessian on top of the stable became soaked and the water was soon drawn evenly over all four sides by the hessian before being finally led away through the drain pipe in the guttering. The relative humidity within the stable rose to about 90 per cent. in a comparatively short time.

A strong box for the horse, 6 feet 6 inches long, 2 feet 6 inches wide, and only 3 feet 6 inches high, so as to render the back of the animal easily accessible, was firmly fixed to the floor near the centre of the stable. It was provided with a manger at the front end. Access to the box was provided for by the end opposite the manger being loose and hinged at the bottom so that it could be lowered and form a " walk in " to the box. This end was long enough to reach through the large door and over the guttering, which it protected from damage by the horses' hoofs, when lowered.

Between the sides of the box and those of the stable a working space of 3 feet on the one side and 2 feet on the other was left.

A fair amount of time was taken with the designing and construction of the stable with the result that it was only completed towards the end of March. From then on it was used continuously, combined with the saddle attachment for the small cages, and in the remainder of the experiments the feeding results were in all cases perfectly satisfactory.

(*g*) Times at which Experiments were carried out.

The mosquitoes were generally put on to the horses in the late afternoon, left on the animals overnight, and taken off again the following morning. Most of the mosquitoes used were night biting species and they had thus ample opportunity for feeding within their hours of predilection. From time to time, especially when large numbers had to be handled, they were also fed during the day-time. In most cases the results were not quite as good as during the night, but some species, especially *Aëdes caballus*, fed almost equally well during day-time.

(*h*) Feeding Results with Different Mosquito Species.

The different species responded differently to the opportunities for feeding offered them by means of the cage method. However, when suitable conditions were provided, every species could be induced to feed.

The easiest to feed was certainly *Aëdes caballus*. Quite regularly 90 per cent. were engorged in a relatively short time and they took up comparatively large amounts of blood. It was not even necessary to starve them beforehand as, often in cases where the abdomen was distended with sugar water, they still readily took up blood.

Aëdes hirsutus was almost as easy to feed as *A. caballus* and this species also took up a large amount of blood.

Aëdes lineatopennis occasionally presented difficulties, although on the whole the results could be regarded as satisfactory. The amount of blood taken up was generally less than in the case of the preceding species.

Aëdes vittatus also fed readily.

Culex theileri gave no difficulty at all and quite a large amount of blood was taken up by this species.

The few *Anophelines* used engorged themselves satisfactorily.

Mucidus scatophagoides was only tried towards the end of the season and, although previously regarded as a non-bloodsucking species, it was found to feed and take up large amounts of blood very readily.

4. METHODS OF FEEDING MOSQUITOES ON SHEEP.

For feeding the mosquitoes on sheep the small wire cages covered with mosquito netting, already described, were used in all cases. The liberation of mosquitoes in fly proof stables was not attempted, as no good results were expected.

To attach the cages to the sheep, the wool was carefully clipped, as close to the skin as possible, from an area on the back of the sheep corresponding to the one surface of the cage. The cage was then placed in position on this area and six pieces of tape, attached to the wool around the clipped area, two on each of the long sides and one on each of the short ends, tied together over it, served to hold it in position. In order to provide good contact and thereby facilitate the feeding of the mosquitoes, it is necessary that the tapes be pulled taut until the skin under the lock of wool to which each is attached is slightly lifted. Up to four cages can be placed on one sheep in this manner, two on either side of the mid line.

At first it was thought that conditions in these cages, which were in direct contact with the skin and surrounded on four sides by thick wool, would be suitable for the feeding of the mosquitoes, but it was soon found that, under our climatic conditions, the mortality amongst them was considerable, even though only placed on the sheep for a few hours at a time. Insufficient humidity was found to be the factor involved, and, in order to rectify this deficiency, a method, which proved both simple and effective, was devised. Before placing the cages in position the surface remote from the skin was covered with a piece of damp cotton wool which was held in position by the tapes passing over the cage. This had one obvious disadvantage of course, viz., that the mosquitoes could possibly take up water from the cotton wool, which was in close contact with the netting, and there is no doubt that in certain cases, where the mosquitoes failed to engorge, this did actually occur. In the greater majority of cases, however, the results were satisfactory.

As our stable for mosquito feeding, with special arrangements for obtaining a humid atmosphere, was mostly in use for horse-sickness experiments, the feeding on sheep had to be conducted in the ordinary sheep stables. On very dry nights the cotton wool occasionally dried up, resulting in a more or less high mortality amongst the mosquitoes, but under normal conditions, the water contained in the cotton wool was sufficient to last out the night.

By this method of mosquito feeding it was not necessary to isolate individual sheep as the cages are not interfered with by other sheep which may be present in the same box. Furthermore, the sheep used were apparently in no way irritated by the presence of the cage(s) as no attempt was made to rub it (or them) off, or, at any rate in our experiments, no sheep succeeded in dislodging a cage.

The feeding results on the whole were not so good as was the case in the horsesickness experiments. The percentage of mosquitoes which engorged themselves, and furthermore, the amount of blood taken up by the individuals was definitely less, whereas the mortality amongst the unfed specimens was certainly higher. A number of reasons may be advanced to account for this fact. The pressure exerted on the cage, on which depended its contact with the skin, was not so great as in the case of the cages used on horses, so that slight movements of the skin, tending to disturb feeding mosquitoes, could not be retarded to the same extent. The regulation of humidity was less effective, and as mentioned before, the mosquitoes had ample opportunity of taking up water. Short wool covering the clipped surface, which was never shaved, mechanically interfered with the insertion of the proboscis, and wool fat present probably also played a part in preventing good feeding.

However, our results were sufficiently satisfactory to permit of our disregarding these minor difficulties.

SUMMARY.

While engaged at Onderstepoort, during the winter of 1931 and summer of 1931-1932, in work on the transmission of horsesickness and bluetongue in sheep by means of mosquitoes, special methods, adapted to the South African climatic conditions, had to be devised.

The obtaining of a sufficiently high degree of humidity, to suit the requirements of the mosquitoes, was the most important factor to be contended with throughout the whole course of the work. The average humidity content of the air in Transvaal is so low that, especially during the summer months, the mosquitoes succumbed in considerable numbers, e.g., when left overnight in cages attached to a horse in one of the ordinary stables.

Larvae and adults were caught in the usual way. Adults of some of the species, e.g., *Aëdes caballus* and *A. lineatopennis*, could be caught from time to time in large numbers on their breeding grounds during the day.

The mosquitoes were kept in the laboratory in small jars or in cages enclosed by mosquito netting. To ensure a sufficiently high degree of humidity, the jars were placed on wet cotton wool in slightly larger jars provided with loosely fitting lids. The cages were kept on shelves in a wooden framework having a waterproof metal top covered above and on the sides by hessian kept wet constantly by a flow of water. By this means favourable humid conditions were provided within the cages in a manner similar to that employed in the larger mosquito cages previously described.

For feeding mosquitoes on infected or susceptible animals, small wire cages, covered by mosquito netting and firmly attached to a clipped area on the animal's skin, were used.

Of all the methods utilized, the best results, so far as attaching the cages to horses was concerned, were obtained by the use of a specially constructed metal saddle fixed to the horse in the usual manner. This saddle contained rectangular openings into which the cages just fitted, being held in position and firmly pressed against the skin of the horse by means of rubber bands.

For use on sheep the cages were held in position on the skin by means of pieces of tape attached to the wool around a clipped area in which these cages just fitted and tied together over each cage. A sufficiently high degree of humidity within the cages was obtained by placing damp cotton wool over each.

In order to obtain the humid conditions necessary for feeding mosquitoes on horses, a special stable was constructed consisting of a wooden framework with a metal roof, covered on the four sides by mosquito netting and having a further outer covering of hessian which was kept wet throughout the duration of each experiment by water distributed over the roof through a small garden spray. This stable contained a strong wooden box just large enough to accommodate the experimental horse, and guttering, for leading off waste water, together with electric lights, for night work, completed the equipment. The dimensions of the stable, viz., 11 feet by 8 feet by 7 feet 6 inches, were such as to allow of ample working space around the horse box. A sufficiently high degree of humidity, e.g., up to 90 per cent., could quite easily be maintained inside the stable.

With the use of the small mosquito cages held in position on horses by the special saddle under favourable conditions of humidity as provided for by the above-mentioned specially designed stable, and furthermore, feeding the mosquitoes at night, very satisfactory results were obtained.