INSTRUCTIONS TO COLLECTORS OF SPECIMENS FOR THE CENTRAL MALARIA BUREAU.

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THE following simple instructions are circulated for the use of those who desire to assist in furthering the study of malaria in India. All material collected should be addressed to-

The Officer in Charge,

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Central Malaria Bureau,

Kasauli.

L-DIRECTIONS FOR COLLECTING AND FORWARD-ING MOSQUITOES.

- 1. Specimens of any kind of mosquito are of value, in whatever condition they may be forwarded.
- 2. As many specimens as possible should be sent.
- 3. Specimens of the common species in each locality are desired, in addition to specimens of new or rare species.
- 4. Specimens of anopheles mosquitoes are particularly desired.

Collecting .- In order to catch adult mosquitoes one needs only a few test tubes (about half an inch in diameter) and some cotton wool. If no test tube is at hand, a small dry bottle or even a tumbler or wine glass will answer the purpose. In the early morning examine the outside of your mosquito curtains and search the walls of bathrooms, outhouses, and stables. In the houses of villages and bazaars select an unoccupied rather dark room and search the rafters of the ceiling and the cobwebs in the corners. Mosquitoes will usually be found resting in these 5 S. C.



places. When one of them is seen to be resting on a suitable (flat) surface; place the mouth of the tube or bottle *slowly* over it. When the mosquito has flown into the tube or bottle, squeeze a plug of cotton wool (or if a tumbler or wine glass is being used, slide a piece of stiff card board or paper) between the mouth of the tube and the surface on which the mosquito was resting. Do not remove the tube from the surface until the escape of the mosquito has been efficiently prevented.

Search for *anopheles* mosquitoes should especially be made; they often look like little white thorns hanging from the ceiling or rafters.

In the jungle mosquitoes may often be caught in test tubes if one sits down quietly under a tree and waits until they rest on one's hand to feed; or they may be found resting on the walls and curtains of one's tent. It is *usually* a waste of energy to attempt to catch mosquitoes while they are flying, but in certain circumstances the endeavour is not to be despised; good catches even of *anopheles* have sometimes been made by beating bushes or heaps of straw, and at the same time sweeping a wide-mouthed net through the air over them. The best net-bags are those made from tulle.

To obtain anopheles by breeding them out from larvæ (which is usually the best method of collecting) their larvæ should be sought (1) by stirring up likely pools so as to make the water muddy and to wash the larvæ out from the edges where they are seen with difficulty, and then dipping the larvæ out with a spoon as soon as they appear on the surface; or (2) by dipping up cupfu's of water from among aquatic weeds or grass fringing the edge of the water and examining to see if one or more larvæ eventually come to the surface. They can then be removed with a spoon to a bottle or tube. All kinds of water should be examined by one or other of these methods until experience has taught the collector the best kinds



Interesting species are likely to occur in unexpected collections of water such as may be found in a hole in the trunk of tree, in the stump of a bamboo, in the axil of a leaf, in the hollow of a rock, in crab-holes, pools on the sea-shore, etc. The edges of small streams and the pools in the bed of a river should always be searched with great thoroughness.

Larvæ are best carried home in small tubes, as they get less shaken in them than in a larger jar or bottle.

Fully grown larvæ should be transferred with some of the water to a glass jar, the mouth of which should then be covered with a piece of mosquito netting or muslin. In a few days the larvæ will change into nymphs and finally hatch out as adult mosquitoes. Much time will have been saved if a number of nymphs have been collected. For the quick development of *anopheles* larvæ bright light is desirable, but if the jar is placed in direct sunlight, the water usually becomes so heated that the larvæ are killed.

Mosquitoes that have hatched out should not be killed immediately; they should be allowed to remain in the bottle for a few hours until their proper colour and hardness have been attained. A clean dry bottle should then be placed mouth downwards over the breeding-out jar and the netting slipped off. Some of the mosquitoes will fly upwards into the empty jar, and then a piece of cardboard should be slipped between the mouths of the jars to prevent the mosquitoes from returning to the lower jar. Then a second piece of cardboard should be slipped over the first piece, so that one can remove the upper jar and leave the lower one still covered. The mosjuitees should be killed by hanging in the jar a small piece of cotton wool moistened with chloroform. The plug of wool should not be dropped into the jar, but should be hung by a thread and pulled out when the mosquitoes are stupefied. Tobacco smoke should be used for killing mosquitoes only when no better killing agent is available.

Preserving, Mounting, Packing, etc.—Immediately the mosquitoes are dead, they should be turned out upon a clean sheet of paper and dealt with at once according to one or other of the following ways :—

A .-- DIRECTIONS RELATING TO UNMOUNTED SPECIMENS.

First method .- Taking up the sheet of paper on which the mosquitoes have been turned out, and using a pin or needle to move the mosquitoes along, push one mosquito over the edge of the paper into a test tube. Push in after the mosquito a small plug of cotton wool pressing this down until it just lightly holds the mosquito in place (be particularly careful not to squash or crush down the mosquito). Tilt in another mosquito and insert a second plug; continue to do this until the tube is full. Packed in this way a test tube will hold from four to six mosquitoes. Lengths of glass tubing $\frac{1}{3}''$ to $\frac{1}{2}''$ diameter can be used instead of test tubes, or soft crumpled paper may be used instead of cotton wool. Do not paste any label upon the outside of the tube, but write the necessary particulars on a small piece of paper, crumple this up, and place in the tube. The most important information required is (a) the locality; (b) the date of capture; (c) conditions under which it is caught, as for example " resting on mosquito net " or " bred from pool in the bed of stream etc.;" (d) collector's name and address.

Pack a number of the tubes properly protected in a box; a thin strip of cotton wool or tow wrapped spirally round the tube forms a very efficient method of protection. Do not hermetically seal the tubes as this induces the growth of moulds.

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Second method.—Tease out a small piece of cotton wool into a light web like mass. Spread it over the bottom of a pill box or match box. Tilt in off the sheet of paper half a dozen or so mosquitoes, distributing them evenly and not too closely together over the wool. Very lightly cover these with another loose piece of wool and close up the box. Tight packing must be strictly avoided. When dealing with large numbers of mosquitoes, a larger box or tin may be used, but this should not be so large as to allow of the wool shaking together durin; transit. Write the particulars as above on the outside of the box, and pack it in a larger box for sending by post.

Third method^{*}.—Take a rectangular piece of tissue paper or other soft paper $3'' \times 2\frac{1}{4}''$ and write on it all necessary particulars. Then fold the paper as shown in figs. 1 and 2. Place the thumb of the right hand into the pocket thus made, and with the finger and thumb of the left hand firmly twist the apex so that a rigid pocket is formed (fig. 3). Place one insect in the pocket and fold over the upper edge (fig. 4). Place one or two packets in a match box and pack this in a larger box, so that it may not be damaged in the post.

B.—DIRECTIONS FOR MOUNTING MOSQUITOES IN A MANNER SUITABLE FOR MUSEUM SPECIMENS.

The following are necessary :---

Fine silver entomological pins, Nos. 19 or 20.

Larger pins, No. 2, or these may be replaced by ordinary pins of at least 11/2 length.

A blunt forceps.

Bristol board or some thick paper or cubes of pith

Some form of store box lined with pith or cork ; or corked specimen tubes, $3'' \times 1$."

^{*} For the details of this method and of the drawings illustrating it (figs. 1 to 4) acknowledgment is made to the "Instructions to collectors" issued by the Entomological Research Committee (Tropical Africa).

Entomological pins and good store boxes can be obtained from the Army and Navy Stores or Lawrence and Mayo, Bombay, but arrangements will be made to supply necessary equipment to collectors who wish to send mounted specimens.

Method.—Cut a square or round card disc about half an inch in diameter. Placing this upon a cork or rubber surface, pick up a fine pin in the forceps, and forcing down the pin, drive the point through the disc of cardboard. Then by using the thumb and fingers, or the interval between the blades of a forceps, drive the pin two-thirds of its length through the card. If the pin's point is turned by the cardboard, or if it is found impossible or difficult to drive the fine pins through this, make a fine prick in the first instance with a needle; but do not make this so large that the fine pin will not be tightly grasped by the cardboard (fig. 5a.)

With a touch or two of a pin or needle turn a mosquito upon its back. Then holding the pin in the forceps, drive the point as straight as possible between the origin of the legs into the thorax; when the pin is felt to meet the cork or rubber surface lift up the mosquito, and (with the greatest care, and merely touching with the needle), arrange the wings and legs roughly in position. This should be done as quickly as possible after death, as if mosquitoes become dry (which they are apt to do very quickly in hot dry weather) it becomes very difficult to arrange their parts without damaging them. When the wings have been opened and the legs satisfactorily arranged, drive a large pin through the disc near its edge, and place in a store box or other safe place to dry. The large pin also serves to carry slips of paper on which particulars regarding the specimen are written.

In driving the large pin through the disc there is danger of damaging or of disarranging the mosquito, and therefore some observers prefer to do this before dealing with the mosquito



at all. In this method a number of discs are first prepared having the fine pin driven through the centre of the disc in one direction and a large pin driven through near the edge in a reverse direction (fig. 5b). The head of the large pin in this case should be pushed up close to the cardboard, so that it does not get in the way later on when the fine pin held near its head by the forceps is being used to transfix the upturned mosquito.

When the mosquito is thoroughly dry, drive the large pin into the cork of a specimen tube and then insert the cork into the tube, so that the mosquito lies protected from all harm within the tube (fig. 6).

Some species when dry are so brittle that the least jar causes damage to them. It is advisable, therefore, finally to wrap round the tube with wool kept in place by a piece of string or tape.

V hen store boxes are used for the despatch of specimens, it is essential—

- (1) That every pin be firmly pressed home, since one loose specimen may wreck the whole. In pressing home a pin always hold it firmly below the card disc and as near the point as possible.
- (2) That the discs are prevented by extra pins from swinging round and so damaging neighbouring specimens, or are given sufficient space to allow of their swinging around without damage. It is well to bear in mind that a disc which seems at the time to be quite tightly fixed on the pin may, especially in a drier climate, become loose enough to swing round.
- (3) That the box should be packed in a much larger outer box with a large amount of soft packing material.

The following modification in mounting may be employed after some practice since it gives specimens less distorted by drying than the method described.

After mounting by the above method, draw down the fine pin until the mosquito actually rests on the cardboard. (The pin will now be quite loose, and a little too much pull will make it drop out altogether leaving the mosquito lying upon the disc). Slip one or, if necessary, two small strips of Bristol board under the abdomen, and when thoroughly dry push up the pin again and allow the small pieces of cardboard to fall away.

C .--- INSTRUCTIONS FOR FORWARDING LARVE.

Place the larvæ in a small dish of water and cover with a piece of blotting paper soaked in formalin, so that the larvæ may be killed by the vapour. When the larvæ are dead, add a little formalin to the water and mix carefully, so that the larvæ are still left floating at the surface. Leave for twenty-four hours. Transfer by means of a spoon to a small tube and push in some crumpled paper. This will prevent the larvæ being shaken about, and preserve the palmate hairs and other delicate structures from damage. See that the tube is about twothirds filled with formalin solution (10 per cent.) and cork tightly. To label write with pencil on a piece of paper, orumple this up and place in the tube. When there are no facilities for carrying out the above method, larvæ should be placed in 10 per cent. formalin and packed as described. Culex larvæ may be placed direct in 10 per cent. formalin.

II.-BLOOD FILMS.

The following will be much valued as a help towards the formation of type collections :---

- 1. Good slides showing any form of parasite.
- 2. Slides shewing what appear to be unusual or peculiar forms of parasite.



3. Slides (stained or unstained) sent as representative^k of the infection in any particular district. These specimens may be-

- (a) those made from bazaar children.
- (b) Those taken in hospital from patients known or suspected to have malarial parasites in their blood.
- 4. Slides (stained or unstained) of splenic or hepatic blood whether from malaria cases, cases of Kala Azar or any other case of splenomegaly.
- 5. Smears of organs, especially liver or spleen, no matter what the case may be.
- 6. Pieces of tissue, especially tissues of malaria cases or of Kala Azar. Slides or tissues from cases of pernicious malaria are especially valuable.
- 7. Slides of any protozoan parasites whether from man or animals, especially blood parasites.

Any specimens utilised for the permanent collection will be labelled with the sender's name, and all possible return will be made in the way of sending named forms of parasite, named types of *anopheles* and so on.

Slides are best forwarded wrapped in paper and then packed in wool or tow in a tin or box, or placed back to back in small boxes which will be supplied on request by the bureau.

If the slides are being sent unstained, they should also be sent "unfixed," as they retain their staining properties better so. Slides should not be put up in hermetically sealed cases. 5 S.C. Tissues are best forwarded in alcohol. Small fragments about the size of a large pea may simply be dropped into a tube containing absolute alcohol, or small slabs may be placed upon a cover glass and then dropped in.

To take blood films .- The following are required :-

- 1. Slides.
- 2. A needle.
- 3. A box to hold slides. The most suitable for many reasons are small boxes to hold about twentyfive slides in an upright position.

It is important that the slides used in taking films should be as clean as possible.

It is best to place a number in hot water and then clean and polish each with a *perfectly clean* handkerchief. In making a good film a really clean slide is half the battle. For pricking the finger and making a film in the manner to be described, a surgical needle is perhaps the best, but a No. 2 entomological pin held with the head end between the fingers makes a good spreader and may be used to prick the finger. In choosing a needle see that it does not bulge at the eye. A thick rigid needle is bad, a flat flexible needle is the best.

If the finger of the patient is obviously dirty, and especially if damp with sweat, it should be roughly wiped with a cloth.

The last phalanx of the finger (the third finger of the right hand will be found most convenient and the skin usually soft) is taken between the finger and thumb of the left hand of the operator and gently pressed to force the blood towards the pulp. A slight prick with the triangular pointed needle will cause a fair-sized drop of blood to exude.



When the drop of blood reaches the size of the head of a pin, a slide is taken in the right hand and lowered on to the drop (taking care not to ' dab ' it on the skin). If the drop is too large, wipe it away and squeeze a small fresh one. The drop should be transferred to the slide about one-third of an inchfrom the far end. The slide is then changed to the left hand, the finger and thumb grasping the end nearest to the drop. With the right hand again take up the needle, and holding it by the pointed end lay the shaft transversely to the slide and across the drop of blood. After waiting about a second, that is, until the drop spreads to the extent of about one-third inch between the slide and needle, draw the needle evenly and not too quickly to the right and so along the whole length of the slide. The correct amount of pressure is very quickly learnt. Immediately the film is made, it should be waved to and fro until it is seen to be quite dry. The quicker the film dries, the more perfectly preserved will be the red cells of the blood.

The following are useful hints :--

1. Do not leave unfixed slides exposed on the flat, as a single fly may in a very few seconds damage it irretrievably. Fly marks are small round clear spaces in the film made where the proboscis has been laid.

2. To label, write with a pencil or needle on a thick portion of the film; or previously number the slides with a writing diamond. (A useful writing diamond can be obtained for about Rs. 7 from Thomson and Company, Calcutta.)

3. If films do not stain well it may be that fixation is at fault. If the strength of the alcohol used is doubtful, leave the slides several hours for fixation.

Leishman's stain should be made up freshly, as in the heat of the plains it rapidly deteriorates (sometimes in one or two days).



Romanowski's stain can always be easily made up according to the following formula :--

Stock solution a-

Methylene blue (medicinal, Grübler)	a seed	1	gramme.
Sodium carbonate (pure)		5	
Distilled water	u (100	10

Place in the sun for four or five days until a strong purple red colour is visible on shaking.

Stock solution b.--Eosin Köchst B. A. (Grübler) 1 in 7000 d'stilled water.

For use dilute each stock solution 25 times.

Use equal quantities of each poured over the slide.

Films should be stained for about 15 minutes.

If after staining with Romanowski the film is bluish, the slide should be allowed to stand in water until the blue has been washed out and the film is nearly or quite colourless.

III.-INFORMATION.

The officer in charge of the bureau will be glad to receive communications on the following subjects :--

- 1. Recording cases of Blackwater fever.
- 2. Noting areas in which malaria seems to be unusually severe.
- 3. Figures giving the results of examinations for enlargement of the spleen.
- 4. Information about the presence of Kala Azar and oriental sore.



To obtain the spleen rate.—As nearly as possible children between the ages of 2 and 10 years should be selected. Young babies and youths should be omitted. A sufficient number of young children can usually be obtained by rewarding them with pice or sweetmeats. As regards number "as many as possible" is the best rule. However small the numbers may be, the results from different villages should be detailed separately. It should always be noted whether the children have been obtained from a school or not.

If possible, the information should be given in the manner of the following statement :--

VillageThanaDistri	ct	mo No.	of child	lren
1. Spleen not palpable			19	
2. Spleen palpable or one finger's costal margin	breadth	below	22	
3. Spleen two to three finger's	breadth	below	57	
4. Spleen a hand's breadth.			8	
5. Spleen to umbilicus or beyond	•••		2	
	Total		108	

Source of the children bazaar.

If an observer is not familiar with palpation he may nevertheless sometimes give information of value as regards the degree of prevalence of the large size spleens, *i.e.*, Nos. 4 and 5 or possibly only No. 5. In obtaining such information, however, care should be taken to exclude those children who are brought forward by their parents or friends because they are known to have enlarged spleens.

The following are useful hints :--

1. Stand facing the person whose spleen is being palpated, with the left hand or his right shoulder, and whilst asking some simple question to engage the person's attention (this is not always necessary) gently pass the fingers over the left



fostal margin on the abdomen. If the spleen is not felt in this way, press gently inwards and upwards so as to explore with one or two movements of the hand the whole left subcostal area.

Some experienced observers palpate by placing the fingers over the costal arch and pressing directly inwards with the thumb. This method, in which one strikes the edge of the organ, is especially useful in palpating the spleen in adults.

If the abdomen is rigid, tell the person to look directly upwards, the abdomen will usually be felt to become quite lax as he does so.

If doubtful as to whether a spleen is palpable or not (especially in the case of village children) it is better to omit the case than to prolong the examination unduly.

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