METHODS AND MATERIALS

1. The test insect.

The common Indian housefly, *Musca nebulo* Fabricius was selected as the test insect primarily because of the ease with which it can be cultured under laboratory conditions throughout the year and its extreme public health importance in this country. It has also a shorter life-cycle in comparison to many other insects and can be reared in abundance for experimental purposes.

*Musca nebulo* can be easily distinguished from *Musca domestica* by its smaller size, narrower thorax bands and lighter colouration of the abdomen.

2. Rearing techniques.

(i) General: Houseflies can be easily reared under laboratory conditions on various media. Hutchison (1916) recommended horse manure for rearing *Musca domestica*. Horse manure mixed with hog manure was suggested by Hockenyos (1931), while Musham (1944) obtained best breeding conditions with cow dung. Pig dung was used by Lorincz and Makara in 1935 and Busvine (1953) reared houseflies on monkey faeces.
Hafez (1948) successfully reared *Musca domestica* on cotton wool soaked in milk diluted with water in a ratio of 3:1. During the present studies the same method with slight modifications was used for rearing *Musca nebulo* at a temperature varying between 27°C and 28°C. Milk was diluted with equal quantity of water and cotton wool pads were soaked in this mixture. Adult flies were kept in sleeve cages (Figure 1) and were fed on these pads. A few cubes of cane sugar were also placed in each cage. The flies readily oviposited on the milk pads, which were replaced by fresh pads after every twenty four hours. The eggs thus obtained were seeded in culture jars containing the food prepared in the manner described above.

The early development of the larvae was greatly dependent on the moisture contents of the medium. It was found that moisture content of 85% to 95% was most favourable for larval development.

The above method of rearing houseflies is undoubtedly one of the best techniques yet developed. In the first place, the medium can be easily prepared in a short time and its constituents milk, cotton and water are easily available everywhere. Again, if the
Figure 1. Cages used for rearing *Musca nebulo*.
containers are washed regularly, mould formation and parasitisation by other flies can be easily checked. No special types of containers are needed and all that is required is to cover the containers with cheese-cloth to prevent the larvae from escaping. There is no need to change the medium or to add fresh milk in the culture and this greatly facilitates rearing.

When the larvae were about to pupate some dry cotton wool was added to the culture in order to facilitate pupation and also to check the larvae from going out of the jars.

Newly formed pupae were removed in small glass vials and were placed in sleeve cages containing a few sugar cubes. A few experiments were also performed in which no sugar cubes were provided to the emerging flies and in all such cases the flies obtained were comparatively unhealthy and inactive and died within two days of emergence. It seems, therefore, that abundance of sugar is absolutely essential for the normal growth of Musca nebulo.

(ii) Rearing cages: Six inches square cages constructed of wire frames covered with loosely fitted cloth sleeves were used for rearing the flies. Such cages are not
only easy to take and handle but occupy much smaller area in comparison to wire gauze wooden cages usually employed for this purpose. Also there is no danger of contamination, as the sleeves can be regularly washed. The same type of cages, but only four inches square in size were used for rearing individual families during crossing experiments.

(iii) Testing cages: Two types of cages were used for testing the insecticides. In preliminary experiments, the flies were exposed to insecticidal residues in cardboard cages, six inches high and five inches in diameter, (Figure 2). Each cage was covered with cheese-cloth on open ends and only one test was performed in each cage, in order to avoid any possibility of contamination.

Cellophane cages, four inches high and 1.5 inches in diameter and having cardboard tops and bottoms (Figure 3) were used for placing treated flies. Such cages have a great advantage over other types of containers which have been previously used for this purpose in that they can be discarded after each test, thereby eliminating all chances of contamination. Again, they are quite cheap and easy to make and handle.
Figure 2. Cardboard cages used for exposing the flies to insecticidal residues.
(iv) Containers for rearing larvae: Glass jars 6x3.4 inches and 6x2 inches in size were used for culturing the larvae. The jars were covered by thick cloth pieces in order to prevent the larvae from escaping.

(v) Feeding dishes: Small petri dishes with a diameter of 3.5 inches each were used for placing cotton wool pads.

3. Method of testing insecticides.

In preliminary experiments, the inside surfaces of cardboard cages (figure 2) were coated with various insecticidal formulations by means of cotton wool pads. Every care was taken to ensure homogeneous coating in the cage. Four days old flies, 200 to 300 in number, were released in each cage and were fed on sugar cubes and diluted milk. Mortality counts were made after twenty four hours of exposing the flies to insecticidal residues.

In topical application method four days old flies were treated individually with an insecticide solution in acetone or in oil. The flies were swept in a test tube and were given a slight dose of carbon dioxide, generated in a small 250 CC. Kipp's apparatus by the action of hydrochloric acid on marble chips. The flies thus anaesthetized were placed on a piece of paper and each fly held by its wings with a fine forceps was
brought to the tip of the needle. A measured drop of the insecticide solution to be tested was placed on the desired part of each fly, the size of the drop being controlled by means of a screw-gauge fitted against the head of the syringe. The flies were soaked while treating and those belonging to the same sex were kept in a collodion cage (Figure 3). Mortality counts were made after twenty-four hours of treatments.

The stock emulsions used during the preliminary tests in cardboard cages were a 25% DDT-kerosene oil emulsion obtained from Goigy Insecticide Ltd., Bombay; a 20% Lindane oil concentrate and a 20% Pyrethrum-Piperonyl butoxide emulsion supplied by the Bombay Chemicals Ltd., Bombay. The various formulations used were prepared by diluting the stock emulsions with desired quantities of water.

In topical application tests 1% stock solutions of Aldrin, Dieldrin, and gamma BHC were prepared from a 98% technical Aldrin and a 80% technical Dieldrin supplied by the Shell Insecticides, and a 14% Lindane (98% pure gamma BHC powder) obtained from the Bombay Chemicals Ltd., Bombay.

Both volatile and non-volatile formulations were tried, acetone being the solvent used in volatile solutions and Risolle oil in non-volatile preparations.
Figure 3. Cellophane cages used for keeping treated flies.
Figure 4. Topical application apparatus:
(a) Assembled apparatus (b) The apparatus in parts.
A few tests were also performed with distilled kerosene oil, Pine oil and Liquid paraffin to find out their values as nontoxic solvents, but the results obtained showed that these chemicals were considerably toxic to *Musca nebulo* and therefore can not be used as solvents in experiments where it is desired to find out the resistance of the flies to some particular insecticide alone.

**Topical application device:** Manipulation of insecticide solutions by means of a fine wire loop was tried in 1949 by Wilson, whereas micropipettes were used by Dresden and Oppenooth (1953) for the same purpose. The idea of a microsyringe for placing liquid drops on insect body was first put forward by Buck (1949) and Hopf (1951) constructed an apparatus in which a hypodermic syringe of moderately fine bore containing the liquid to be applied was mounted rigidly on a stand, with the needle pointing downwards. The plunger was depressed by means of micrometer screw head which was turned through a reduction worn gear and in this way minute and readily varied drops of liquid were obtained. The drop obtained was, however, much too small to fall unaided and was blown downwards of the needle tip by a tuft of air.
through a special fitting.

During the present tests a tuberculine syringe 'IYT' with a fine needle of uniform bore was used. The syringe was partially filled with the insecticidal solution to be tested and was held in between the two pieces of a metallic holder fitted in the 'U' of a screw gauge (Figure 4) in such a way that the head of the screw gauge was in direct contact with the piston of the syringe. The screw gauge together with the syringe was then mounted on a stand so as to keep the syringe horizontal during the experiments.

As only one syringe was available for tests with different insecticides, extreme care was taken to avoid all possibilities of contamination and the syringe was carefully washed with acetone both before and after a particular test. Thus constant drops of insecticide solutions could be placed on the desired parts of the flies by bringing an anaesthetised fly near the tip of the needle and rotating the head of the screw through five divisions on the circular scale.

This method of topical application of insecticides
is undoubtedly one of the simplest techniques yet evolved. The apparatus can be easily assembled in the laboratory at a very low cost and its component parts - micrometer screw gauge and the syringe are easily obtainable. It is simple, handy, and portable, as well as accurate. But perhaps its great advantage lies in the fact that a large number of insects can be treated with it in a much shorter time than is required while working with similar other devices.