A NEW RADIOACTIVE METHOD FOR MARKING MOSQUITOES

Mario B. Aragão
Instituto de Malariologia do S.N.M.
and
Elisa Frota Pessôa and Neusa Margem
Centro Brasileiro de Pesquisas Físicas e Faculdade Nacional de Filosofia
Rio de Janeiro, D.F.
August, 1952

A technique used in the study of animal migrations consists of marking a determined number of specimens, freeing them and by means of subsequent captures, locating their path.

For obtaining the range of the mosquitoes' flight, the following technique has been utilized: the insects were taken in large numbers from nature; they were dusted with powder (the type used for painting metalic colors), freed at a determined point and, by means of captures at diverse distances, the range of the migration was determined [1].

Mosquitoes were bred in a solution of radioactive isotopes, such as phosphorus, the later identification being made with the help of counters [2].

Lattes [3] suggested the possibility of identifying the radioactive mosquitoes by the employment of nuclear emulsion. This method, of much higher sensibility than that of counters, permits the use, even in small quantities, of long-lived radioactive elements like thorium and uranium for marking the mosquitoes.

This has been the method used in the present work, in which two techniques were employed: the use of prepared plates, and the use of liquid emulsion for the preparation of the plates containing the material to be studied. The second method has the advantage of per-

mitting the simultaneous observation of the preparation and of the tracks of alfa particles from the radioactive substance.

In the first case, the material was used on a glass slide and placed in contact with the face of the plate on which was the emulsion. In the second case, the material was glued to a glass slide and covered with the liquid emulsion.

In either case, the whole thing was kept for several days in the refrigerator. After the exposure, the plates were developed and fixed.

The prepared plates used were type G-5, (Ilford), of 50 micra, and the liquid emulsion was type G-5. For the detection of alfa particles, as was the case, we could have used emulsions less sensitive than G-5.

Results of Observation

When the mosquito was left entire, no tracks of alfa particles were obtained in the emulsion. This would indicate that the thorium localized internally, and the alfas were absorbed before reaching the emulsion.

The exoskeleton was removed, that is to say, the mosquito dissected in order to obtain tracks of alfa particles. It was observed in the plates convered with liquid emulsion that the thorium was not evenly distributed throughout the material. In plates where the various organs of the same mosquito were separated, it was verified that the thorium was found in the Malpighian tubes.

The ashes of five mosquitoes of the adult phase, two months old, raised in a solution of $\frac{1}{100.000}$ thorium nitrate, still contained thorium, as observed by the method of prepared plates.

Control mosquitoes, both those taken from nature and those raised in the laboratory, did not produce any alfa particle tracks in the emulsion.

Fig. 1 shows part of a scraping of the Malpighian tubes and of the genital apparatus, covered with liquid emulsion. The paths of the alfa particles are clearly visible. The exposure took 17 days. The mosquito had 12 days of life in the adult phase and was bred in

a $\frac{1}{20.000}$ solution of thorium nitrate.

Fig. 2 shows a microscope field which contains part of the Malpighian tube of a female Anopheles (Kerteszia)sp. It shows tracks of alfa particles which originated in this organ. The mosquito was enclosed in parafin and the sections, of three micra, were glued to the slide. To eliminate the parafin, the slide was submerged for 10 minutes in Xilol and for 10 minutes in 100% alcohol. After drying, the plates were covered with liquid emulsion, then developed, fixed and dyed with hematoxeline through the emulsion. The exposure time was 29 days, the mosquito had 20 days of life in the adult phase, and its breeding was effected in a $\frac{1}{20.000}$ solution of thorium nitrate.

Breeding of the Mosquitoes

The work was accomplished quite as well with mosquitoes from a natural breeding ground as with those from the water collected in bromeliads. The latter developed well even in a solution of $\frac{1}{10.000}$, while the former could not support a concentration higher than $\frac{1}{50.000}$. The salt used was thorium nitrate.

The solution in which the larvae were bred was prepared with water from the same type of focus where the larvae breed in nature. As supplementary nourishment, the larvae received corn meal and granulated Fleishmann's yeast. The adults were kept in wire cages, covered with damp towels, and fed with honey and human blood.

Preparation of Plates Covered with Liquid Emulsion

The slides with mosquitoes were arranged on a level plane, so that the liquid would be evenly distributed, and over them was poured the liquid emulsion at 51° C. In order that the emulsion should reach clear to the edge of the slides, the operation was furthered with a stainless steel spatula, in accordance with Ilford's instructions. For a slide with a surface of 18 cm^2, approximately 1.6 gr of emulsion were used.

Development and Fixation of Plates

The following developer was used[4]:

distilled water -- 1,000 cm^3

boric acid -- 35 gr
sodium sulphite (anhydrous) -- 18 gr
potassium bromide (10% solution) 8 cm^3
amidol -- 4.5 gr

The developing time was 20 minutes, at a temperature of 28º C.

Stop bath:

0.2% acetic acid solution

The plates were put into the stop bath at an initial temperature of 28º C, which was gradually lowered to 5º C, and kept there for one hour.

Fixing bath (according to the process recommended in photographic technique):

water -- 1,000 cm^3
sodium hyposulphite -- 400 gr

After fixing, the plates should be washed for two hours in water at 10º C, which is renewed each half-hour.

Grateful acknowledgment is made to Professor G. M. de Oliveira Castro for his helpful discussions; to zoologist Rudolf Barth for executing the sections, and to Mr. Luiz Machado de Lima for the photographs.

1. Majid, J.S.A.M. Rec. Mal. Surv. Ind., 7, 105, 1937.

2. Hassett, C.C. and D.W. Jenkins, Science, 110, 109, 1949.

3. Private communication.

4. Dilworth, C.C., G. Occhialini et L. Vermaesen, Notes du Centro de Phys. Nucl. de Bruxelles, Nº 13A, Fevrier, 1950.

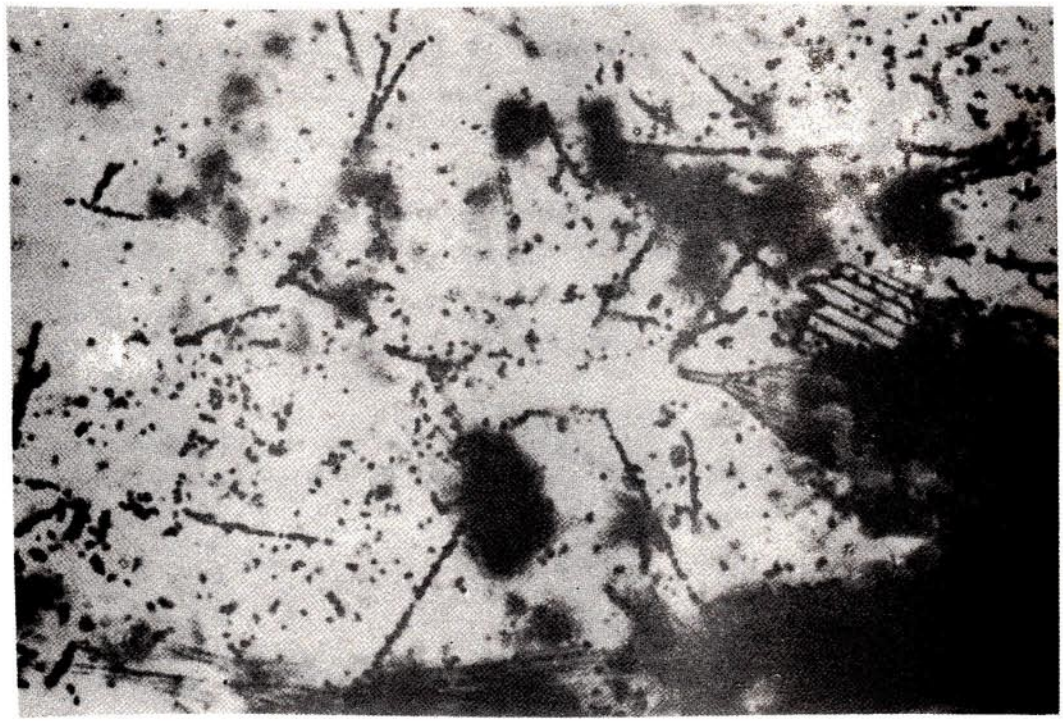

Figure I

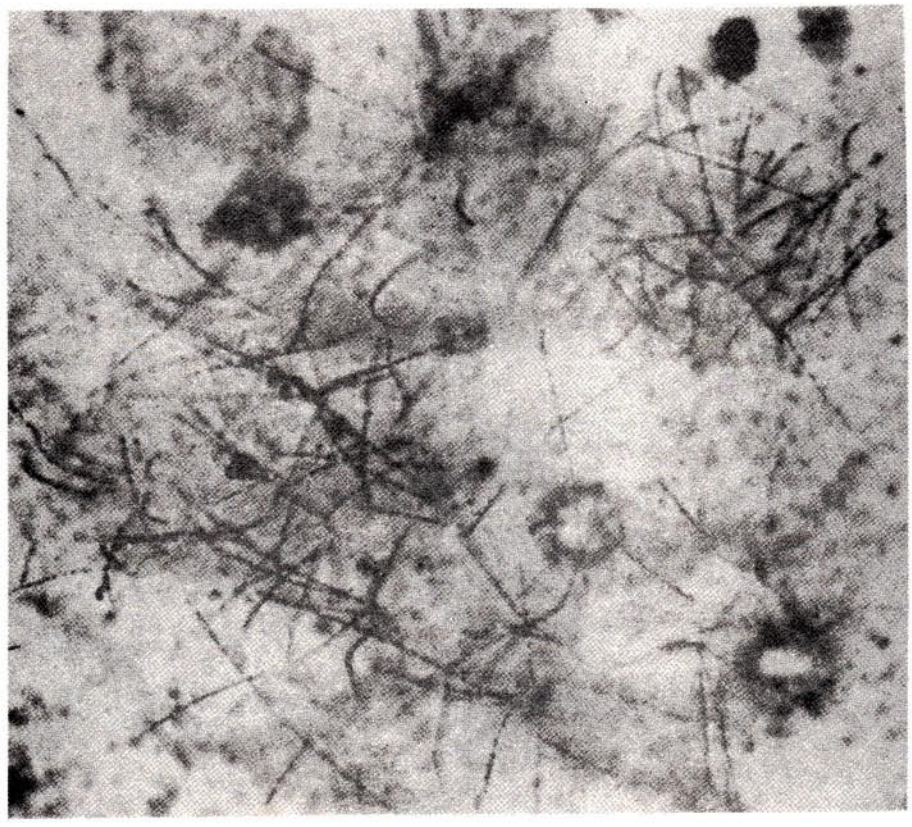

Figure II