

# ARTIFICIAL FEEDING OF IXODID TICKS FOR STUDIES ON THE TRANSMISSION OF DISEASE AGENTS

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Various artificial feeding techniques have been used in studies on the nutritional requirements of haematophagous arthropods and on their capability as vectors of infectious disease agents. The objective of most of these techniques is to feed a particular arthropod upon normal or infectious liquids, usually blood, through various types of membranes. Thus, mosquitoes, flies, fleas, bugs, lice and even ticks have been fed through artificial membranes or through membranes prepared from the skins of mice, rats, rabbits, chickens and other animals.\*

In studies on the behavior of pathogenic microorganisms in ticks, argasid ticks were fed successfully upon the air sac membrane of embryonated chicken eggs (Burgdorfer and Pickens, 1954). This technique was also used for experimental infection of *Ornithodoros turicata* with *Leptospira pomona* (Burgdorfer, 1956). The results of the later study showed that leptospires persist and multiply rapidly in tissues of this tick, which is a capable experimental vector of the organism (Burgdorfer, 1956). Since the possible role of ticks in the epizootiology of *Lept. pomona* infections, especially in cattle, horses and pigs, had not been explored, the observations with the argasid tick, *O. turicata*, led to a series of experiments in which the ixodid ticks, *Dermacentor andersoni* and *Amblyomma maculatum*,

both common parasites of livestock, were examined for their ability to ingest, preserve and transmit leptospires. Unfortunately, these ticks could not be induced to engorge either upon the air sac membranes of infectious chicken eggs or through mouse skin. Attempts were then made to infect them by use of a modification of the glass capillary tube technique developed by Chabaud (1950) for artificial feeding of the ixodid ticks *Hyalomma excavatum*, *H. dromedarii*, *Dermacentor pictus* and *Rhipicephalus sanguineus*. This technique, which proved to be simple and successful, is applied as follows:

Ticks to be infected are fixed by slight pressure to a ridge of plasticine on an ordinary microscope slide and are immobilized by pressing small clumps of plasticine over their mid and hind legs. Glass capillary tubes, 35.0×1.1 mm, with one end drawn to suitable diameter, are then filled by capillary action with infectious suspensions, and the fine ends of the glass tubes are pushed over the hypostome and cheliceral elements of the ticks with the aid of low power magnification (fig. 1 and 2). Blocks of plasticine are used to hold the capillaries in position and as operating tables for the manipulation of the tubes.

Using this technique, 75 *D. andersoni* and 25 *A. maculatum*, males and females, were induced to ingest a suspension of *Lept. pomona* in Verwoort's medium. The ticks fed readily at room temperature for 4 to 6 hours, during which period they ingested from 0.01 to 0.03 ml of the suspension as measured

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\* A review of the most important references was recently presented by Kartman (1954) in his description of an apparatus for the experimental feeding of fleas.



FIG. 1.—tubes.




FIG. 2.—chelicerae

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pipettes.  
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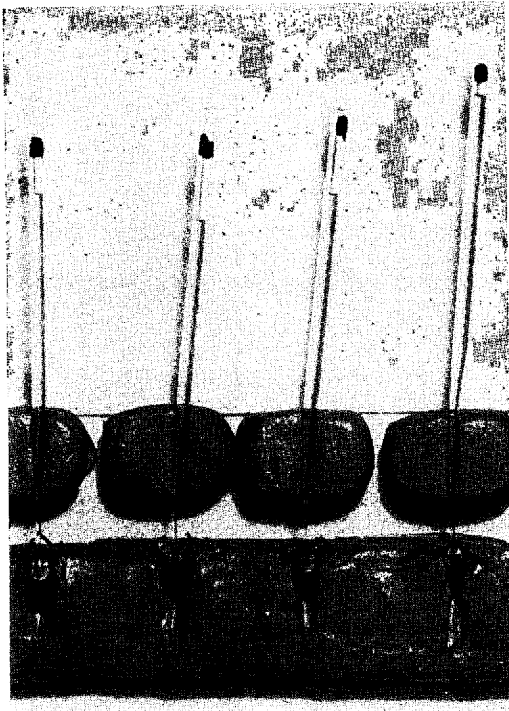


FIG. 1.—*D. andersoni* feeding from capillary tubes.



FIG. 2.—*D. andersoni* with hypostome and chelicerae inserted into capillary tube.

and calculated by means of serological pipettes. Further feeding activities were seldom observed after that time unless

fresh suspensions were offered. In no instance was it possible to obtain fully engorged ticks. The partially fed ticks were then removed and stored in desiccator jars for further tests. About 14 days later, transmission experiments were carried out by feeding the ticks on weanling guinea pigs. All ticks completed engorgement, and several of the test animals became infected with *Lept. pomona*.

The same technique was also successfully applied in studies on the possible role of ixodid ticks in the epizootiology of rabies. *D. andersoni* adults were fed by this means on an infectious suspension prepared from mouse brains triturated in defibrinated rabbit blood. Data on behavior of rabies virus in ticks will be the subject of a separate paper by Bell et al.

#### DISCUSSION

The described feeding technique provides an excellent artifice for experimental infection of ixodid ticks with viruses or other pathogens. To a great extent it eliminates the use of expensive laboratory animals which in the past had to serve as blood donors for the infection of these arthropods. Because of irregular feeding habits of the Ixodidae, it is impossible to obtain uniformly infected ticks for experimental studies, a difficulty which can be overcome by use of the technique here described. Known concentrations of culture suspensions of microorganisms can be fed to ticks, thus providing the basis for obtaining accurate data on the development of pathogens in tick tissues. This technique, furthermore, is of great value in studies of the transmission of disease agents. In the past, ixodid ticks infected in the nymphal stage could not be tested for their ability to transmit pathogens until several weeks after their development to adults.

By the capillary tube technique, ticks can be infected within a few hours and after a brief incubation, transmission experiments can be carried out by placing the ticks upon laboratory animals to complete their engorgement.

## SUMMARY

A technique for the artificial feeding of ixodid ticks for studies on the transmission of infectious agents is described. The method consists of placing glass capillary tubes filled with infectious fluid over the hypostome and chelicerae of ticks immobilized in plasticine. The ticks ingest up to 0.03 ml of fluid during a feeding period of 4 to 6 hours. The technique proved suitable for experimentally infecting *Dermacentor ander-*

*soni* and *Amblyomma maculatum* with *Leptospira pomona* and *Dermacentor andersoni* with rabies virus.

## REFERENCES

- Bell, J. F., Burgdorfer, W. and Moore, G. J. The behavior of rabies virus in ticks. *J. Infect. Dis.* **100**: 278-283.
- Burgdorfer, W. 1956, The possible role of ticks as vectors of leptospirae. I. Transmission of *Leptospira pomona* by the argasid tick, *Ornithodoros turicata* and the persistence of this organism in its tissue. *Exper. Parasitol.* **5**: 571-579.
- Burgdorfer, W. and Pickens, E. G. 1954, A technique employing embryonated chicken eggs for the infection of argasid ticks with *Coxiella burnetii*, *Bacterium tularensis*, *Leptospira icterohaemorrhagiae*, and western equine encephalitis virus. *J. Infect. Dis.* **94**: 84-89.
- Chabaud, A. G. 1950, Sur la nutrition artificielle des tiques. *Ann. Parasitol.* **25**: 42-47.
- Kartman, L. 1954, Studies on *Pasteurella pestis* in fleas. *Exper. Parasitol.* **3**: 525-537.