

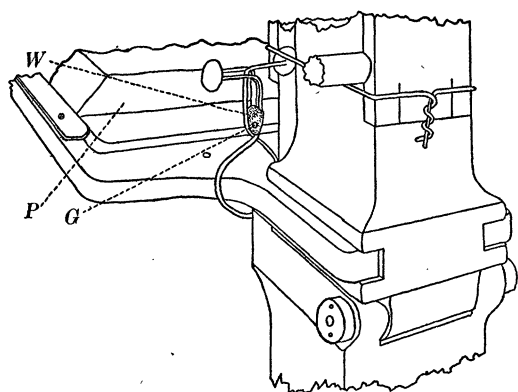


FIG. 2. Portion of binocular microscope showing method of attaching spring clamp for control of gas to microburner. Glass rod (G), held in place by wax (W), keeps clamp partially open. P, special paraffin stage.

slide under the stage-holders (Fig. 2, P). Parawax cakes are of a convenient shape and satisfactory consistency for this purpose. A straight, shallow groove, about two inches long and parallel to the front of the microscope, was cut in the center of the stage.

The injection procedure was as follows.

A microinjector, with bulb not over half filled with the liquid to be injected, was placed in the wire holder. The ring stand, carrying this bulb and the microburner, was placed at the right of the microscope at such a distance that the range of the pipette was over the groove in the paraffin stage. An individual previously anesthetized in one eighth of 1 per cent. chloretone was then transferred to the stage in a medicine dropper. After most of the solution had been removed, the worm was stretched out in the groove

with the tip of its prostomium a short distance from the left end. A microscope slide or cover-glass was then placed over the anterior end of the worm, leaving several segments exposed anterior to the point at which it was planned to make the injection. A cover-glass proved the more satisfactory since its flexibility made it possible to vary the pressure exerted on the worm even though the latter did not quite fill the groove. With a few flashes of the gas flame the bulb was slightly warmed until the solution began to flow slowly in drops from the tip of the pipette. The cover-glass was then held down firmly with one or two fingers of the left hand, while with the right the end of the pipette was carefully inserted into the coelom of the worm and pushed anteriorly three or four segments from the point of entrance. Without removing either hand, the base of the index finger of the left hand was lightly pressed several times against the outer finger grip of the spring clamp to give brief flashes of heat to the bulb. If the bulb was heated too rapidly the side toward the flame became so soft that it could not withstand the pressure created within.

This method has the advantage that worms are held firmly in place while the solution is injected with a minimum of injury. The method of warming the bulb makes it possible to control carefully the flow of the injection-solution while both hands are at the same time occupied with other tasks. In addition the holder is simple, easily made and consequently more readily obtainable and less expensive than the type used by Knower.

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SPECIAL ARTICLES

THE VIRUS OF LARYNGOTRACHEITIS OF FOWLS

INFECTION laryngotracheitis or infectious bronchitis of fowls has been recognized as a distinct and definite disease since 1924. The disease has become so highly destructive in the United States and Canada that it menaces the poultry industry in certain regions. There is no record of its occurrence in other countries.

The disease is now being studied with material obtained from four sources—two in New Jersey, and two in California. The poultry raising districts in California from which part of the material came have suffered severely from the affection during the past four years.

Bacteriological study having failed to reveal a consistent visible microorganism in association with the lesions of the disease, and spleens and livers, proved

to contain the causative agent, having been bacteriologically sterile, a filterable virus was sought for and found in the inflammatory exudate contained in the tracheas of infected fowls.

Seven filtrations from the exudate have been made through five different Berkefeld "V" filters, and it was shown that infectious material was present in six of the seven filtrates. Of five filtrations made through Berkefeld "N" filters, three proved infectious and two non-infectious. In one of the five instances, the same material passed through a "V" filter was active. As yet no infection has been secured with material passed through Seitz filters. The incubation period of the filtrate inoculations has been the same as that of inoculations of non-filtered suspensions of the exudate.

Experiments on antibodies for the virus are in progress. Complete inactivation or neutralization of the virus has already been obtained when mixed with

serum from each of two recovered fowls, while in other instances the onset of the symptoms of infection has been much delayed.

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THE LONGEVITY OF BACILLUS AMYLOVORUS (BURR.) TREV. IN ASSOCIATION WITH HONEY

IN recent years interest in the part played by meteoric water in the dissemination of the fire blight organism has diverted the attention of some workers from the long-established¹ rôle of pollinating insects as vectors. Gossard and Walton² in 1922 demonstrated that the organism may be found in honey from the beehive and that it may be recovered after 72 hours from artificially infested honey.

The results of the following experiments suggest that a more detailed examination should be made of the relation of the bee and the hive to the fire blight problem.

A heavy bacterial suspension in water was made from the exudate from blighted pear twigs. This was applied with a camel's hair brush to the outside surface of a frame of honey, to the surface of the comb and to the uncovered cells at the margin of the comb. The frame was placed in a covered glass dish without the addition of water and stored in a dark cupboard at laboratory temperatures (Berkeley, California).

Transfers were made to bouillon with flamed instruments at intervals of 3, 5, 7, 9, 11, 13, 15, 20, 30, 40 and 55 days from the time the experiment was started. Inoculations were made directly from the bouillon on green pear fruits in the laboratory or apple shoots in the greenhouse. In this manner the presence of the organism was demonstrated in the honey cells in every attempt up to 15 days but in no instance thereafter. It was recovered from the wood of the frame at 3, 5, 9, 11, 13 and 20 days. From the waxy surface of the comb virulent organisms were obtained in every attempt up to 55 days when the experiment was terminated.

A second experiment was made similar to the first with the following exceptions. Undiluted bacterial exudate from inoculated pear fruits was applied to

the surface of the comb only. The lid of the dish was raised slightly to provide for more ready circulation of air. Vigorous and pathogenic cultures were obtained from this source at 21 and 35 days, after which the experiment was discontinued.

Admittedly the conditions of these tests are artificial and the number of organisms was probably greatly in excess of any number likely to be introduced into the hive by the bees. The matrix in which the organisms are embedded in the exudate apparently affords decided protection of the organisms. The writer has isolated virulent organisms from rather large drops of the exudate on apple twigs, after these had been kept dry in the laboratory for 12 months (at Ithaca, New York). It seems significant, however, that the bacteria remained alive on the surface of the comb 35 days longer than was the case on the surface of wood or in the honey. Although it seems impossible to standardize the samples from these three sources, it is of interest that the growth in bouillon was in most if not all cases more profuse with the sample from the comb than with the others.

Three important questions are involved here:

1. The organisms may be carried from blossoms into the hive and thence to other susceptible plants in the neighborhood.

2. They may be transferred from one locality to another when the bees are moved for the purpose of effecting pollination in orchards. (This appears to be a rather common practice in some districts.)

3. It is possible (though it seems improbable) that the bacteria may occasionally survive in the beehive from the time of the scattered late blossoms in autumn until the first blossoms which appear in the spring.

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THE CLARIFICATION OF PLANT JUICES: NITRATE CONCENTRATION IN LARGE AND SMALL LEAVES

IN view of the increasing interest in the chemical study of the expressed juice of plants, and the recent publications (Hill,² Cook,³ McCool and Weldon,⁴ Holtz and Larson,⁵ Emmert⁶) on the determination of nitrate nitrogen in these juices, it may be of interest to describe advances in a method used in this laboratory (Gilbert⁷) for securing samples of plant

¹ Contribution No. 394 of the R. I. Agricultural Experiment Station.

² H. H. Hill, *SCIENCE*, 71: 540, 1930.

³ R. L. Cook, *Journ. Am. Soc. Agron.*, 22: 393, 1930.

⁴ M. M. McCool and M. D. Weldon, *Journ. Am. Soc. Agron.*, 20: 778, 1928.

⁵ H. F. Holtz and C. Larson, *Plant Physiol.*, 4: 288, 1929.

⁶ E. M. Emmert, *Plant Physiol.*, 4: 519, 1929.

⁷ B. E. Gilbert, *Plant Physiol.*, 1: 191, 1926.

¹ M. B. Waite, "The Life-history and Characteristics of the Pear Blight Germ," *Proc. Amer. Assoc. Adv. Sci.*, 47: 427-8, 1898.

² H. A. Gossard and R. C. Walton, "Dissemination of Fire Blight," *Ohio Agr. Exp. Sta. Bul.* 357, 1922.