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

J. R. BEACH,
Division of Veterinary Science, University of California
DEPARTMENT OF ANIMAL PATHOLOGY,
ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, N. J.

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-established[1] rôle of pollinating insects as vectors. Gossard and Walton[2] 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.

H. E. THOMAS
UNIVERSITY OF CALIFORNIA

[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.

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] Emmert[6]) on the determination of nitrate nitrogen in these juices, it may be of interest to describe advances in a method used in this laboratory (Gilbert[7]) 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.

juice free from substances which interfere with the determination of nitrate nitrogen by the phenoldisulfonic acid method. The greatest modification lies in the elimination of carbon black, certain brands of which are known to adsorb nitrate nitrogen. The simplicity of the method should commend it to workers in the field of plant chemistry. The use of carbon black was found to be unnecessary since the colloidal complex formed by the reaction of the $Ca(OH)_2$ with the $CuSO_4$ and $AgSO_4$ adsorbs all the coloring matter present in plant juices.

Place a sample of plant tissue sufficient to yield approximately 10 cc of juice in a cheesecloth bag and freeze thoroughly for at least two hours. This is best done by solid CO_2, although an ice-salt bath may be used, if sufficient time is allowed for thorough freezing. Remove from refrigerating medium, thaw and press at once in any device which will give sufficient mechanical pressure. A hydraulic press of small size is extremely convenient for this expression, since it is essential that the pressure be the same on all samples if a comparison of the results is to be made. Collect the expressed juice, centrifuge to remove any solid material, and pipette a suitable aliquot (usually 2 cc) into a 100 cc volumetric flask.

To the juice in the flask add about 20 cc of nitrate-free distilled water; 5 cc of saturated $AgSO_4$ solution; 1 cc of N $CuSO_4$ solution, and 0.2 gram of finely divided $Ca(OH)_2$, shaking after each addition. These reagents should be thoroughly tested for the presence of nitrate nitrogen, and only those showing an absence of nitrates should be used. Make to volume with nitrate-free water, and filter after standing at least one hour. Discard the first portion of the filtrate. A suitable aliquot of this can be evaporated to dryness on a steam bath, and the nitrate-nitrogen determined by the phenoldisulfonic acid method, using NaOH to neutralize the acid according to Harper.[8] If a precipitate forms at this point, allow to flocculate and filter.

A detailed study of the method above proposed has been published.[9]

In connection with some determinations of the nitrate nitrogen in the juices of the beet plant, a significant negative correlation appeared between the weight of the leaves and the nitrate nitrogen found in the juice of these leaves. Upon further study, the correlation calculated from a statistical study of 578 individual leaves divided into twelve composite samples indicated a correlation of -0.855 ± 0.052. An explanation of this may be found in the following facts:

(1) The juice of the midrib of the beet leaf contains more nitrate than the juice from the remainder of the leaf.

(2) The ratio of the weight of leaf tissue after removal of midrib to the weight of midrib is much higher in the large leaves.

These facts offer an explanation for the correlation, for, since the high nitrate juice is in the midrib, and the proportion of the weight of midrib to the weight of the remainder of the leaf is greater in the small leaf than in the large, it is obvious that the smaller leaf should have a higher nitrate content.

In view of the above facts, it has become the practice in this laboratory to choose leaves of uniform size and to remove the midrib of these leaves before the extraction of juice for analysis. It is hardly probable that this entirely eliminates the error attributed to the size of the leaves, since considerable venous tissue remains, but it is felt that this error is much less when the midribs are discarded.

A more complete study of the above facts has been prepared, and will be published soon.

DONALD E. FREAR

R. I. AGRICULTURAL EXPERIMENT STATION

[8] H. J. Harper, *Journ. Ind. Eng. Chem.*, 16: 180, 1924.
[9] D. E. Frear, *Plant Physiol.*, 5: 359, 1930.

THE GEOLOGICAL BACKGROUND OF PEKING MAN (SINANTHROPUS)[1]

THE *Sinanthropus* discoveries are significant for a number of reasons, quite apart from the valuable evidence they present regarding the close relatives of the anthropoid stock from which the genus *Homo* evolved. (The anatomical facts themselves are made available through the reports issued periodically by Dr. Davidson Black from the laboratory of Cenozoic Research in Peiping. Therefore, beyond brief comments on the cast of the skull and on the photographic reproductions thrown on the screen, this short outline of the salient points deals with other aspects of the discoveries, which are reported officially in the publications of the Geological Society of China.)

(1) The story of the discovery at Chou-kou-tien offers a striking example of the romance of scientific research, beginning with Andersson's studies of the Tertiary and Quaternary history of North China, and his search for fossiliferous deposits (1921), followed by Zdansky's careful paleontological work on the mammalian remains (1922), and the finding of the first teeth when the material was being prepared in the laboratory at Upsala (1926); continued by Bohlin's methodical excavation leading to the unearthing of the single tooth on which Black based the new

[1] Abstract of paper presented at the meeting of the British Association, Bristol, by permission of the director of the Geological Survey of China.