by him. No inoculation experiments were made, for the reason that sufficient cultures in suitable condition could not be obtained from any medium then known.

Since the media used in former investigations are not suitable for obtaining cultures for purposes of inoculation, in taking up the further study it has been necessary to devise a medium which would be satisfactory in this respect. Such a medium has been discovered, and large amounts of the culture suitable for experimental inoculations have been obtained. This medium is prepared and used as follows: Healthy bee-larvae or young pupae are picked from the comb, crushed, strained through cheese cloth, diluted with 20 to 50 times their volume of water, filtered through ordinary filter paper, and then passed through an earthenware filter (the Berkefeld filter is satisfactory) to remove any bacteria which are present. The sterile filtrate thus obtained may be pipetted into tubes or flasks and stored until needed. When Bacillus larvae is to be isolated, a tube of the ordinary agar of the laboratory is liquified and cooled to 45 degrees or 50 degrees C. Then about 2 c.c of the filtrate mentioned above is added to it. A very small amount of the decaying larvae affected with American foul brood is then added. The procedure from this point is as usual in making agar plate cultures; these plates are afterward incubated. When a large amount of culture is desired for experimental purposes it is convenient to use the ordinary agar medium in large test tubes to which has been added, as above, about 2 c.c. of the sterile larvae filtrate. These agar tubes are then inclined and the surface of the congealed agar is inoculated. In no case should the larvae or filtrate reach a high temperature. The object, of course, is to obtain a medium which contains the food constituents which are afforded the bacteria in the living larvae.

Inoculation experiments have been made by feeding to a healthy colony the scales from combs which had contained brood affected with American foul brood. The result of the feeding was that the colony became affected by disease, the symptoms of which were the same as those observed in the apiary where American foul brood is found. Like symptoms have been produced by feeding scales which had been put into ordinary meat bouillon incubated for 24 hours, and then heated to 65 degrees C for twenty minutes.

On microscopic examination of the decaying larvae dead from the disease thus produced experimentally, the same large number of spores and rods are seen as when samples are examined which are taken from apiary affected with American foul brood. From these dead larvae pure cultures of bacillus larvae were obtained from plates, using the new medium described above. These experiments show that by the feeding method the disease may be produced and that the contagion is found in the scales. The second experiment tends to indicate that the cause of American foul brood as found in the scale is not killed by heat at 65 degrees C. applied for 20 minutes.

Up to the present time there is no authentic record of this disease having been produced by experimental inoculations of pure cultures.

Knowing that by the feeding method the disease may be produced, pure cultures of bacillus larvae have been mixed with sterile sugar syrup and fed to healthy colonies with the result that the disease appeared in the colonies within three weeks with symptoms identical with those produced by feeding the scales of the disease. In the ropy brown mass which is produced experi-

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