AMERICAN MUSEUM NOVITATES

Number 304 — Published by The American Museum of Natural History, New York City — March 29, 1928

59.57,99:11.05

ENZYMES AND BACTERIA IN THE HONEY BEE

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While reviewing the literature of the physiology of the digestive system of the honey bee (*Apis mellifera*) my attention was attracted to the varying results in the studies of the enzymes entailed in digestion. For an instance, Petersen (1912) lists three: diastase, invertase, and a proteolytic enzyme. Pavlovsky and Zarin (1922) list nine: catalase, inulase, lactase, invertase, lipase, pepsin, trypsin, chymosin and emulsin. Of these it is rather difficult to understand any reason for the presence of diastase, lactase, and possibly chymosin as native digestive ferments.

Diastase is an agent in the digestion of starch. In this process dextrins are formed as intermediates and they are poisonous to honey bees, being the cause of bee dysentery. Lactase reduces only lactose, a purely animal sugar found in milk, and I doubt its being a part of a bee's diet. Chymosin's rôle in digestion is to coagulate proteins, as those found in milk. It may have a doubtful claim based on the possibility of the protein in pollen needing its action, or the bare possibility of a minute quantity of protein being present in nectar and needing it. Catalase is in another category and it is very doubtful if it plays any part in digestion. Its sole purpose is to reduce the peroxides formed during metabolism and thus prevent the death of the tissue by excessive oxidation. It is present in small quantities in all living tissue. However, the presence of large quantities of this enzyme in the digestive tract will be accounted for below.

Petersen's finding of diastase may be accounted for. In animal tissues a diastase-like enzyme, glycogenase, is found for the conversion of stored animal starch, glycogen, into glucose, its utilizable form. The remaining enzymes, invertase, trypsin, lipase, etc., are necessary for the reduction of the higher sugars, as sucrose to glucose, the digestion of protein in pollen and the emulsifying and digesting of the fats in pollen. The presence of the extraneous enzymes may be explained as being produced by the bacteria so plentiful throughout the digestive system.

At the American Museum's Station for the Study of Insects, Tuxedo, New York, we examined the flora of the digestive tracts of seventy-two presumably normal healthy honey bees. Our procedure was as follows:

1.—Bees were captured arriving at and leaving the hive and killed with cyanide fumes.

2.—They were then immediately dissected in a sterile Petri dish with sterile instruments observing all precautions for preserving an uncontaminated excised digestive tract.

3.—The digestive tract was washed in sterile physiological salt solution to free it externally of body fluids and then cut in four sections—proventriculus, ventriculus, small intestine and rectum.

4.—Each of these sections was used to inoculate a tube of sterile 0.5% glucose broth.

5.—These were incubated for twenty-four hours at room temperature and then plated on three media—plain nutrient agar, 0.5% glucose agar, and eosin-methylene blue agar.

6.—These plates were then incubated four days at room temperature and the various types of colonies on each examined and tube cultures made on plain nutrient agar and, when necessary, 0.5% glucose agar.

7.—Single cell cultures of each tube were then made for identification purposes.

In all, over two hundred cultures were isolated and the diagnostic reactions of each recorded. Grouping like cultures together, we accepted as specific thirty-four strains representing four families of Eubacteria.

Coccaceæ	2
Spirillaceæ	2
Bacteriaceæ	25
Bacillaceæ	5
	34

Of these, ten had been recovered by earlier investigators of the flora of bees. Distributing them according to the location from which they were isolated we find:

	Proventriculus	Ventriculus	Small Intestine	Rectum
Coccaceæ	1	2	0	0
Spirillaceæ	0	1	0	1
Bacteriaceæ	3	2	11	10
Bacillaceæ	1	3	0	1
	5	8	11	12

The strains represent the following genera:

Coccaceæ	
Streptococcus	1
Micrococcus	1
Spirillaceæ	
Spirillum	1
Vibrio	1
Bacteriaceæ	
Achromobacter	3
Flavobacterium	2
Lactobacillus	1
Salmonella	2
Eberthellia	9
Proteus	4
Escherichia	2
Aerobacter	2
Bacillaceæ	
Bacillus	5

A future paper will fully describe the various strains and compare them with named strains isolated and described by other workers in bee and general bacteriology.

The enzymes produced by these bacteria included all of those that have been listed from bee digestive tracts. Diastase, however, was generated only in traces. It is probable that when a bee becomes infected with a diastase producer and the hydrolysis of the starch in the pollen by that bacteria produces dextrin it causes the disease called bee dysentery. No complete study of the several anaerobic strains isolated has been made, but as a group there is little difference in the enzymes produced, only one enzyme being lacking among anaerobes that is present in aerobes, that is catalase. Without its presence a bacterium cannot exist aerobically since the production of peroxide takes place in the presence of free oxygen and peroxide is detrimental to the organism (McLeod and Gordon, 1923, 1925). The fact that aerobes produce great quantities of catalase probably accounts for the presence of it in the rectum, noted by Pavlovsky and Zarin especially after the winter hibernation when all the wastes of that period have accumulated including the wastes of the bacteria.

In order to test our deductions that many enzymes reported were extraneous, we repeated the experiments of previous writers to determine the presence of digestive enzymes. The only changes made in their procedures were to make sure that little or no bacterial products were included in our materials. This was done by carefully splitting the digestive tract and washing it free of its contents with sterile saline solution before grinding it up. The sterility of the triturated masses was tested by inoculating a small portion into 0.5% glucose broth. Tubes showing marked bacterial growth in twenty-four hours were considered to indicate contaminated masses. All tubes showed some growth but in most cases this was negligible. These masses gave uniform positive reactions for only three digests listed above, namely, invertase and the two proteolytic enzymes, pepsin and trypsin. Lipase was recorded doubtfully once and positive once in five tests. It is recognized that lower organisms may adapt themselves to produce enzymes to suit their food. It may be that bees do so to some degree, and, for an example, produce inulase when called upon to utilize inulin for food, although the experiments of Phillips (1924) seem to indicate to the contrary.

CONCLUSIONS

1.—Care must be taken in work on the enzymes of the digestive tracts of insects to exclude those produced by micro-organisms present.

2.—Much of the literature on the digestion of honey bees at least must be read and quoted with that fact in mind.

3.—Normal intracellular enzymes similar to digestive enzymes must be recognized.

4.—The normal native enzymes of the digestive system of honey bees are an invertase, both peptic and tryptic proteolytic enzymes, and a lipase.

5.—Other carbohydrases may be present under special conditions.

6.—One cause of bee dysentery is probably an infection of diastase producing bacteria.

I wish to acknowledge gratefully the assistance accorded me by Dr. Edward Rushmore of the Tuxedo Memorial Hospital, Tuxedo, New York, in allowing me the use of his laboratory, and by Miss Margaret Stevens, his technician, during the early stages of the work. The final bacteriological work was carried on in the laboratory of the Newport Hospital, Newport, R. I., where I was greatly assisted by Miss Hazel Heffron, the technician in charge. To a far greater degree I am indebted to Dr. Frank E. Lutz in charge of The American Museum of Natural History's Station for the Study of Insects at Tuxedo, New York, for many helpful suggestions and friendly interest during my stay at the Station.

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