

THE UTILIZATION OF CARBOHYDRATES AS FOOD BY HONEYBEE LARVAE¹

By L. M. BERTHOLF²

*Formerly Junior Biologist in Apiculture, Bee Culture Investigations, Bureau of Entomology, United States Department of Agriculture*³

INTRODUCTION

The food of honeybee larvae, as of animals in general, consists of mixtures of carbohydrates, proteins, fats, mineral salts, water, and presumably vitamins. But, of course, this statement does not mean that the larvae can utilize all the carbohydrates, all the proteins, all the fats, etc. Thus far, however, no one has reported any attempt to discover which particular ones from each of these classes of foods the larvae can use.

Since carbohydrates constitute one of the most important classes of foods, and since they are for the most part well known chemically, it has seemed to the writer logical to begin the consideration of this problem with an investigation of the ability of the larvae to use carbohydrates as food.

This investigation seemed worth while for two reasons: (1) It might be of practical value to the beekeeper to know which carbohydrates the larvae can utilize, since during the latter part of their larval life workers and drones are fed large quantities of carbohydrates, often furnished by the beekeeper; (2) from the point of view of comparative physiology it would be of interest to know how honeybee larvae compare with honeybee adults, and also with other insect larvae and adults, in their ability to utilize certain carbohydrates.

REVIEW OF PREVIOUS WORK ON DIGESTION OF CARBOHYDRATES IN INSECTS

A review of the literature regarding carbohydrate digestion in insects reveals, as mentioned above, no previous work on honeybee larvae, but shows considerable work on honeybee adults and on larvae and adults of other insects in several of the orders. (Table 1.) Most of the work has been a search for enzymes in the various portions of the alimentary tract. Usually an extract was made of the organ under investigation and the action of this extract was tested on various carbohydrates. According to this method, if a carbohydrate was hydrolyzed by the extract, the enzyme peculiar to that process was supposed to be present.

In a few instances, in the investigations of Cleveland (7),⁴ Sieber and Metalnikow (21), Chapman (6), and Phillips (17), a direct feeding

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³ Now professor of biology, Western Maryland College, Westminster, Md.

⁴ Reference is made by number (italic) to "Literature cited," p. 451.

method was employed, a group of insects being fed on a certain carbohydrate until all of its members died and the length of life of such a group being compared to that of other groups kept as checks. If the group fed on a certain carbohydrate lived longer than a similar, starved group, its members were supposed to possess the ability to utilize that carbohydrate as food.

TABLE 1.—A summary of investigations on the digestion of carbohydrates by insects

Name of insect	Part investigated *	Investigator	Date	Results of tests made for the following enzymes:								
				Amylase	Invertase	Maltase	Lactase	Cellulase	Inulase	Raffinase	Emulsin	Others
Orthoptera:												
Blatta orientalis.	Extract of salivary gland.	Basch (2)	1858	+								
Do.	Extract of walls of mid-gut.	do	1858	+								
Do.	Secretion of salivary glands.	J. de Bellesme (11).	1876	+								
Do.	Secretion of gastric coeca.	do	1876	—								
Do.	Secretion of Malpighian tubes.	do	1876	—								
Do.	Extract of salivary glands.	Swingle (24).	1925	+	—	—	—					
Do.	Extract of fore-gut.	do	1925	—	+	+	—					
Do.	Extract of gastric coeca.	do	1925	—	+	+	—					
Do.	Extract of mid-gut.	do	1925	—	+	+	—					
Do.	Extract of hind-gut.	do	1925	—	—	—	—					
Periplaneta americana.	Pulverized salivary glands.	Plateau (19).	1876	+								
Stethophyma grossum.	Extract of salivary glands.	Plateau (18)	1874	+								
Locusta viridissima.	do	do	1874	+								
Do.	Extract of salivary glands, pupa.	do	1874	+								
Do.	Extract of salivary glands, larva.	do	1874	+								
Isoptera:												
Reticulitermes flavipes.	Ability to live.	Cleveland (7).	1924					—				
Hemiptera:												
Cicada sp.	Extract of whole gut.	Axenfeld (1).	1904		+							
Aphrophora salicis (De Geer).	Extract of salivary glands, larva.	Gruner (10).	1900	+								
Do.	Extract of alimentary tract, larva.	do	1900	—								
Nepa cinerea.	Extract of salivary glands.	Plateau (18)	1874	+								
Ranatra linearis.	do	do	1874	+								
Notonectes sp.	Extract of whole gut.	Axenfeld (1).	1904		+							
Lepidoptera:												
Liparis dispar.	Secretion of mid-gut of larva.	Plateau (18)	1874	+								
Pieris brassicae.	Extract of gut of larva.	Biedermann (4).	1910	+								
Do.	Extract of gut, adult.	Axenfeld (1).	1904		+							
Tineola bisselliella.	Excrement of larva.	Sitowski (22).	1905	(b)				—				
Galleria mellonella.	Ability to live.	Sieber and Metalnikov (21).	1904	—	—							
Do.	Extract of intestine of larva.	do	1904	+								
Do.	Extract of whole feeding larva.	Straus (23)	1909	+	+				—	+		

^a The adult insect is referred to unless otherwise stated.^b Very little.

TABLE 1.—A summary of investigations on the digestion of carbohydrates by insects—Continued

Name of insect	Part investigated	Investigator	Date	Results of tests made for the following enzymes:								
				Amylase	Invertase	Maltase	Lactase	Cellulase	Inulase	Raffinase	Emulsin	Others
Lepidoptera—Continued.												
Euproctis chrysorrhoea.	Extract of whole feeding larva.	Straus (23)	1909	+	+	+	+		?	+		
Do.	Extract of whole pupa.	do	1909	+	+		+		?	+		
Ocneria dispar.	Extract of whole feeding larva.	do	1909	+	+	+				+		(f)
Bombyx neustria.	do.	do	1909	+	+		—			—	+	
Do.	Extract of whole pupa.	do	1909	—?	+	+	—			—	+	
Bombyx mori.	Analysis of food and excrement.	Kellner (12)	1884					—				
Do.	Extract of whole feeding larva.	Straus (23)	1909	+	+		+			+	+	
Do.	Extract of whole not-feeding larva.	do	1909	(g)	+		—			—	—	
Do.	Extract of intestine of larva.	Axenfeld (1)	1904	—	—							+
Do.	Extract of whole pupa, 10 days.	Straus (23)	1909	+	+	+	—			—	+	
Do.	Extract of whole pupa, 14 days.	do	1909	+	+	(h)	—			—	+	
Do.	Extract of whole pupa, 18 days.	do	1909	—	+	—	—			—	+	
Do.	Extract of whole pupa, 21 days.	do	1909	— ⁱ								
Do.	Extract of whole adult.	do	1909	{ ⁻ⁱ + ^h }	+		—			—		
Hypomeuta sp.	Extract of whole feeding larva.	do	1909	+	+	+	+			+	+	
Do.	Extract of whole pupa.	do	1909	+	+	?	—			+	+	
Carpocapsa pomonella.	Extract of gut of larva.	Axenfeld (1)	1904		+							
Vanessa sp.	Extract of gut.	do	1904		+							
Vanessa urticae.	Extract of whole pupa.	Straus (23)	1909			+	—					
Diptera:												
Calliphora vomitoria.	Extract of whole feeding larva.	do	1909	+	+	+	—			—	+	
Do.	Extract of whole not-feeding larva.	do	1909	+	+	—?	—			—	+	
Do.	Extract of whole pupa.	do	1909	+	+	+	—			—	+	
Do.	Extract of whole adult.	do	1909	+	+	+	—			—	+	
Do.	Extract of excrement.	Weinland (25).	1906	—								
Do.	Extract of whole adult.	Weinland (26).	1907	+								
(Musca) Sarcophaga carnaria.	Extract of gut of larva.	Axenfeld (1)	1904		—							
Do.	Extract of gut of adult.	do	1904		+							
Musca domestica.	Extract of whole adult.	Kobert (13)	1903	+						—	—	
Coleoptera:												
May beetles.	do.	do	1903	+								
Tenebrio molitor.	Contents of mid-gut of larva.	Frenzel (9)	1882	+								
Do.	Extract of mid-gut of larva.	Biedermann (3).	1898	+	+	+		—				
Carabus sp.	Extract of whole gut.	Axenfeld (1)	1904		(i)							
Dytiscus dimidiatus.	Fluid in crop of adult.	Plateau (18)	1874	—								
Do.	Extract of mid-gut.	do	1874	—								
Do.	Extract of small intestines.	do	1874	—								

^c Both in gut and in body.^d In the gut; questionably, in the body.^e Question marks in these columns mean doubtfully positive results.^f No agar ferment.^g Little.^h For glycogen diastase.ⁱ For starch diastase.^j Weak.

TABLE 1.—A summary of investigations on the digestion of carbohydrates by insects—Continued

Name of insect	Part investigated	Investigator	Date	Results of tests made for the following enzymes:								
				Amylase	Invertase	Maltase	Lactase	Cellulase	Inulase	Raffinase	Emulsin	Others
Coleoptera—Con.												
Dytiscus sp.	Extract of whole gut	Axenfeld (1)	1904	—	(i)	—	—	—	—	—	—	—
Dytiscus marginalis.	Fluid in crop of adult	Plateau (18)	1874	—	—	—	—	—	—	—	—	—
Phymatodes variabilis.	Extract of mid-gut of larva.	Seillière(20)	1905	—	—	—	—	—	—	—	—	(*)
Tribolium confusum.	Ability to live	Chapman (6)	1924	+	—	—	—	—	—	—	—	—
Oryctes nasicornis.	Extract of mid-gut of adult.	Plateau (18)	1874	+	—	—	—	—	—	—	—	—
Melolontha vulgaris.	do	do	1874	+	—	—	—	—	—	—	—	—
Melolontha sp.	Extract of intestine	Axenfeld(1)	1904	—	(i)	—	—	—	—	—	—	—
Hydrous caraboides.	Fluid in oesophagus	Plateau(18)	1874	+	—	—	—	—	—	—	—	—
Do	Contents of mid-gut	do	1874	+	—	—	—	—	—	—	—	—
Do	Extract of mid-gut wall	do	1874	+	—	—	—	—	—	—	—	—
Hydrophilus piceus.	Fluid in oesophagus	do	1874	+	—	—	—	—	—	—	—	—
Do	Contents of mid-gut	do	1874	+	—	—	—	—	—	—	—	—
Do	Extract of mid-gut wall	do	1874	+	—	—	—	—	—	—	—	—
Hydrophilus sp.	Extract of intestine	Axenfeld (1)	1904	—	+	—	—	—	—	—	—	—
Hymenoptera:												
Ants	Extract of whole pupa	Kobert (13)	1903	+	—	—	—	—	—	—	—	(i)
Apis mellifica	Extract of head	Erlenmeyer (8)	1874	+	+	—	—	—	—	—	—	—
Do	Extract of thorax	do	1874	(i)	(i)	—	—	—	—	—	—	—
Do	Extract of abdomen	do	1874	+	+	—	—	—	—	—	—	—
Do	Extract of fore-gut	Axenfeld (1)	1904	—	+	—	—	—	—	—	—	—
Do	Extract of mid-gut and of hind-gut.	do	1904	—	(i)	—	—	—	—	—	—	—
Do	Known substance fed; analyzed in mid-gut.	Petersen (16)	1912	(i)	+	—	—	—	—	—	—	—
Do	Known substance fed; analyzed in fore-gut.	do	1912	—	+	—	—	—	—	—	—	—
Do	Extract of mid-gut	do	1912	+	—	—	—	—	—	—	—	—
Do	Extract of fore-gut	Pavlovsky and Zarin (15).	1922	—	—	—	—	—	—	—	—	—
Do	Extract of ventriculus	do	1922	+	+	—	—	—	—	—	—	—
Do	Extract of small intestine.	do	1922	—	—	—	—	—	—	—	—	—
Do	Extract of large intestine.	do	1922	—	—	—	—	—	—	—	—	—
Do	Ability to live	Phillips (17)	1924	—	+	+	—	—	—	—	—	(m)

i Weak.

k Xylanase.

l Zymase.

m Melezitase; trehalase.

For the purpose of comparison, the results of the various investigators are brought together and presented in Table 1. This table gives the name of the insect, the part of the insect investigated, the method, the name of the investigator, the year, and a list of all the carbohydrate-digesting enzymes found. The insects are listed under their respective orders, but no attempt is made to classify the insects within an order.

Regarding the use and scope of Table 1 attention should be called to the following facts:

1. The table merely indicates in any case that the enzyme was, or was not, found, and does not usually indicate its quantity or intensity.

2. Negative results mean simply that the investigator tested for the enzyme in question and did not find it. It may be that the enzyme was present in quantities too small to be detected by the test used.

3. In the case of feeding experiments it is assumed that the ability to use a substance as food means that the enzyme necessary to digest that food is present.

4. A host of insects whose feeding habits are well known in a general way, but whose digestive abilities have not been specifically investigated, are for obvious reasons excluded from the table.

One may briefly summarize as follows the results of previous investigations regarding the ability of insects in general to digest carbohydrates:

1. Amylase is very widely distributed among insects, having been demonstrated in nearly every species investigated, but is often localized in a particular organ.

2. In but very few species has the insect been found entirely lacking in any of the common carbohydrate-digesting enzymes, i. e., amylase, invertase, and maltase.

3. No insect investigated has been shown to be able to digest cellulose. It is quite probable, however, that many insects not investigated can do so.

4. In the case of most species every enzyme present is of direct use in digesting the food which the insect eats.

5. With some few species, however, enzymes occur which are probably never used, e. g., amylase in ant pupae and trehalase in honeybees.

6. In the case of a few other species substances are habitually eaten for which the insect secretes no digesting enzymes, e. g., wood by termites.

In regard to honeybees in particular, the review of the literature shows the following facts (see Table 1, *Apis mellifica*):

1. All work of this sort has been done on adults; none on the larvae.

2. All work except Phillips's and part of Petersen's has been done by the method of extracts.

3. All investigators using the method of extracts have found amylase somewhere in the animal, yet Phillips found no ability to utilize starch as food.

4. All investigations have revealed invertase somewhere in the animal.

5. In two investigations lactase, inulase, and emulsin were sought, either directly or indirectly, but were not found.

6. From one investigation it appears that cellulase is not present.

7. From one investigation it appears that bees can readily utilize the rare sugars, melezitose and trehalose.

The problem now is to ascertain how the larvae of honeybees compare with adult honeybees and with other insects in their ability to utilize carbohydrates as food.

MATERIAL AND METHOD

Two methods of investigation were open in approaching this problem, the method of making extracts of various organs and testing the action of these extracts on various carbohydrates, and the method of feeding a group of larvae on a given carbohydrate and ascertaining whether or not the larvae receive any nourishment from it, by comparing the length of life of this group with that of a similar group fed on water only.

The latter method, the direct method, was chosen for two reasons: (1) It enabled one to test any number of carbohydrates in a direct manner, without encountering the technical errors present in many of the chemical tests for carbohydrates; in fact, there are as yet no specific chemical tests for many carbohydrates; (2) the results discovered by direct feeding would seem to be of more practical value than results obtained by the method of extracts, since in the former only those enzymes are considered which are of actual use in keeping the larva alive.

In performing an experiment to compare the length of life on a given food with the length of life on water alone the procedure was as follows: Enough larvae of as uniform size as possible were removed from the comb to serve for the whole experiment. As each larva was taken from the comb with a transferring needle it was floated out on water, where any food clinging to it was washed off. After a sufficient number of larvae had been removed they were divided into lots of 10 to 25 each, and each lot was weighed. The weight was usually about 18 mgm. per larva, although in some experiments the average was as low as 11 mgm. and in others as high as 46 mgm. According to Nelson and Sturtevant (14), the weight of larvae at hatching averages 0.100 mgm.; at 1 day of age, 0.650 mgm.; at 2 days, 4.745 mgm.; at 3 days, 24.626 mgm.; and at 4 days, 93.990 mgm.; it follows that larvae weighing 18 mgm. are, on an average, nearly 3 days old.

After weighing, the larvae were transferred to a small watch glass containing a solution of the carbohydrate to be tested; the watch glass was placed inside a Petri dish containing a little water, to serve as a moist chamber; and the whole was put inside an incubator at about hive temperature (35° C.). An experiment consisted of a number of such lots of similar larvae, fed on various concentrations of the food in question, together with one lot supplied with water only, as a check. For most of the lots examinations were made at intervals of six hours, the dead being removed at this time and the food replaced by a fresh supply if it appeared in the least cloudy. A larva was considered dead if it no longer responded by muscular contraction when touched with the transferring needle. The time at which death occurred was assumed to have been midway between the time of the examination at which it was found dead and the time of the last previous examination. The carbohydrates used were dextrose, levulose, galactose, sucrose, maltose, lactose, trehalose, melezitose, starch, glycogen, and dextrin, in various concentrations, and, finally, a series of honeys. Lots were run in groups of 5 to 10 at a time, and a starvation check, in which distilled water alone was fed, was run with each group. The expressions "starvation check" and "water check" are used interchangeably throughout this paper. Often two or three such groups or series were run for a single carbohydrate.

The complete record of a typical lot, as given in Table 2, will make clear the procedure in each case.

Other lots fed at the same time and under similar conditions had, as average lengths of life, those fed on 5 per cent sucrose, 11.80 ± 0.62 hours; 15 per cent sucrose, 17.98 ± 1.18 hours; 25 per cent sucrose, 32.90 ± 2.95 hours; 50 per cent sucrose, 59.66 ± 4.23 hours; and water, 8.68 ± 0.15 hours.

TABLE 2.—A complete record of all the observations and computations made for a typical lot of honeybee larvae ^a[Lot 83; food, 35 per cent sucrose; ^b weight, 0.0185 gm. each]

Date, 1924	Hour	Number of larvae alive	Number of larvae died since last examination (=n)	Length of life (hours) (=l)	n×l
Aug. 18.	12 m.	20			
Do.	6 p. m.	19	1	3	3
Do.	11.30 p. m.	19			
Aug. 19.	6 a. m.	19			
Do.	12 m.	18	1	21	21
Do.	6 p. m.	18			
Do.	11.30 p. m.	18			
Aug. 20.	6 a. m.	18			
Do.	12 m.	15	3	45	135
Do.	6 p. m.	11	4	51	204
Do.	11.30 p. m.	6	5	57	285
Aug. 21.	6 a. m.	4	2	63	126
Do.	12 m.	2	2	69	138
Do.	6 p. m.	2			
Do.	12 p. m.	1	1	81	81
Aug. 22.	6 a. m.	0	1	87	87
Total			20		1,080

^a All other lots in these experiments were treated in a similar way.^b Thirty-five per cent sucrose means 35 gm. of pure sucrose crystals in 100 c. c. of solution. All solutions mentioned were made in the same way.

Mean length of life, 54 hours.

Probable error of the mean (Peters' formula), 2.39 hours.

It was possible in this way to get a comparison of the effect of any concentration of any carbohydrate desired on the length of life of the larvae.

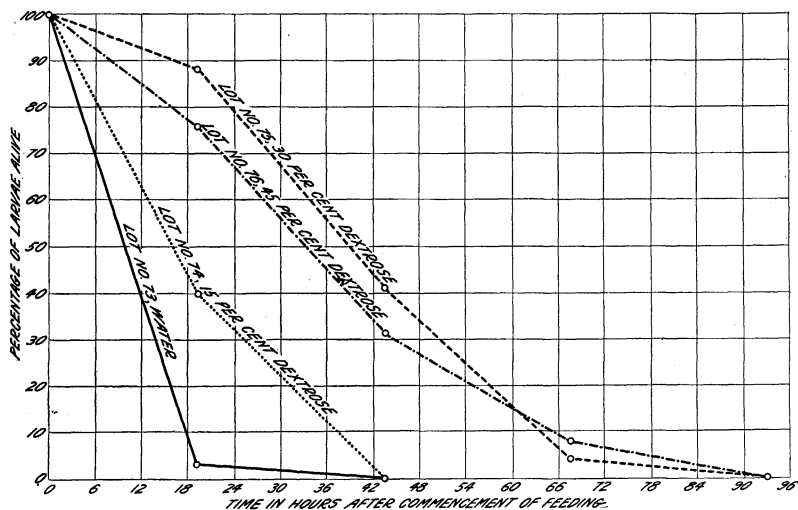


FIG. 1.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of dextrose in certain concentrations with water, and of one lot supplied with water only

The data obtained in these feeding experiments are presented in Tables 3 to 22 and Figures 1 to 11. The experimental results are

given under the headings monosaccharides, disaccharides, trisaccharides, polysaccharides, and honeys. Following these a section

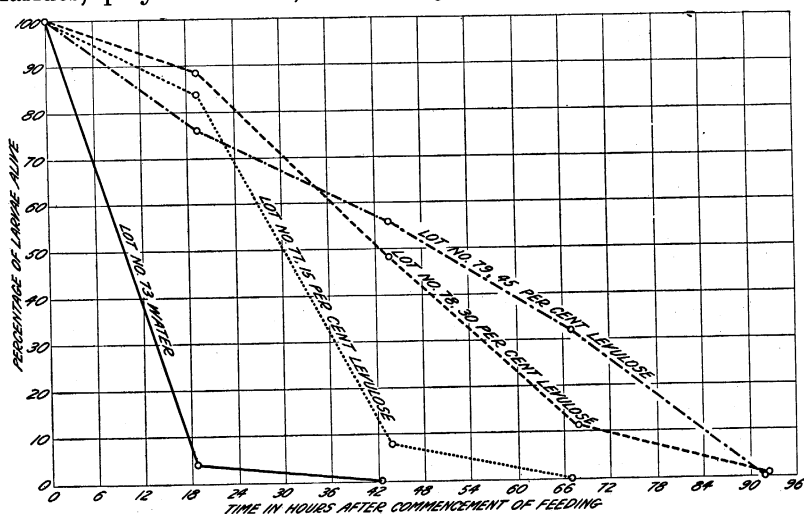


FIG. 2.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of levulose in certain concentrations with water, and of one lot supplied with water only

is devoted to a review and a comparison of those lots, showing optimum length of life for each carbohydrate used.

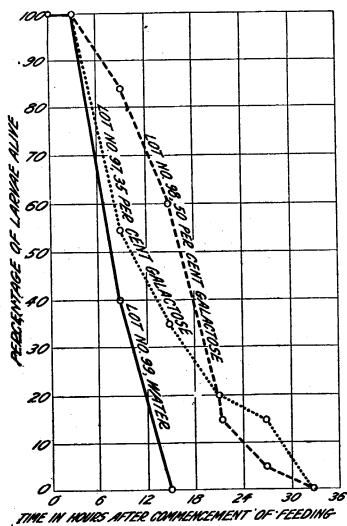


FIG. 3.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of galactose in certain concentrations with water, and of one lot supplied with water only

EXPERIMENTAL RESULTS

MONOSACCHARIDES

Of the monosaccharides, the simplest of the sugars, only the three common representatives were tested—dextrose, levulose, and galactose. All lots fed on dextrose or levulose outlived the larvae fed on water, lot 75, in 30 per cent dextrose, living about four times as long, and lot 79, in 45 per cent levulose, about four and a half times as long, as the lot fed on water (Tables 3, 4, and 5, and figs. 1 and 2). Most of the lots fed on galactose likewise outlived the water check, lot 98, on 50 per cent galactose, living nearly twice as long as lot 99 on water. (Tables 6 and 7 and fig. 3).

It seems evident, then, that the larvae can get nourishment from these simple sugars. The next question is, can the larvae digest other, more complex carbohydrates, changing them to these simple forms? If they sustain themselves on these other carbohydrates it

will be considered evidence that they do digest them; if they die in as short a time as they die when starved, it will appear that they do not digest these carbohydrates.

TABLE 3.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on dextrose in specified concentrations with water, and a lot supplied with water only, as a check* ^a

Lot 73 (water)		Lot 74 (15 per cent)		Lot 75 (30 per cent)		Lot 76 (45 per cent)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>
18.5	4	19.5	40	19.5	88	19.5	76
42.5	0	43.5	0	44.5	40	44.0	32
				67.5	4	67.0	8
				93.0	0	92.5	0

^a Data presented graphically in fig. 1.

TABLE 4.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on levulose in specified concentrations with water, and a lot supplied with water only, as a check* ^a

Lot 73 (water)		Lot 77 (15 per cent)		Lot 78 (30 per cent)		Lot 79 (45 per cent)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>
18.5	4	20.0	84	20.0	88	20.0	76
42.5	0	44.5	8	44.5	48	44.0	56
		67.0	0	67.5	12	67.0	32
				92.5	0	92.0	0

^a Data presented graphically in fig. 2.

TABLE 5.—*Average length of life for lots of larvae fed respectively on solutions of dextrose and of levulose and on water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			<i>Mgm.</i>	<i>Hours</i>
73	Water.....	25	19	10.10±0.55
74	15 per cent dextrose.....	25	18	18.27±1.43
75	30 per cent dextrose.....	25	16	39.90±2.32
76	45 per cent dextrose.....	25	18	36.01±2.73
77	15 per cent levulose.....	25	19	30.57±1.58
78	30 per cent levulose.....	25	18	39.06±2.12
79	45 per cent levulose.....	25	19	47.56±3.64

TABLE 6.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on galactose in specified concentrations with water, and a lot supplied with water only, as a check* ^a

Lot 97 (35 per cent)		Lot 98 (50 per cent)		Lot 99 (water)	
Time	Surviving	Time	Surviving	Time	Surviving
<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>
3.0	100	3.0	100	3.0	100
9.0	55	9.0	85	9.0	40
15.0	35	15.0	60	15.0	0
21.5	20	21.5	15		
27.0	15	27.0	5		
32.5	0	32.5	0		

^a Data presented graphically in fig. 3.

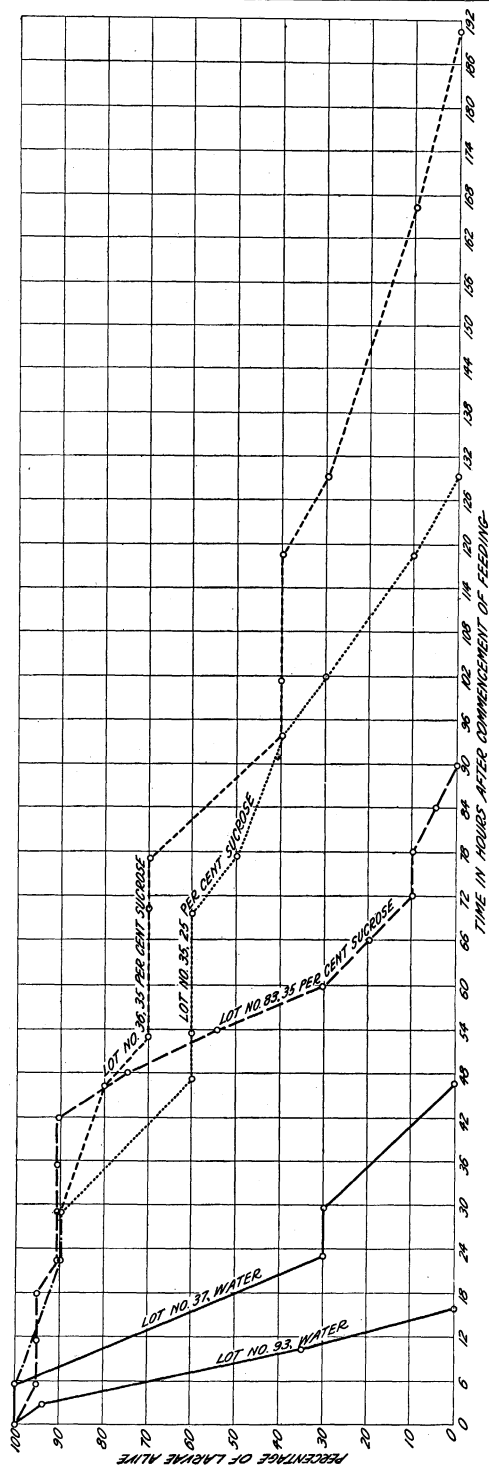


Fig. 4.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of sucrose in certain concentrations with water, and of one lot supplied with water only

DISACCHARIDES

The disaccharides commonly used as food by animals are sucrose, maltose, and lactose. Honeybee larvae were fed on all of these, and also on trehalose, to which particular interest is attached in connection with the honeybee. This rare sugar occurs, so far as known, only in a few fungi. The interest here lies in the fact that Phillips found adult honeybees to be able readily to utilize this sugar as food, in spite of the fact that it probably never occurs in their normal diet.

In the experiments here described the larvae lived longer on all concentrations of sucrose than on water, the concentration of 75 per cent being the most favorable; the ratio of the length of life of lot 125 (75 per cent sucrose) to lot 129 (water) is about 15 to 1, that of lot 84 (50 per cent sucrose) to lot 93 (water) 7 to 1, and that of lot 36 (35 per cent sucrose) to lot 37 (water) $4\frac{1}{2}$ to 1. (Tables 8 and 9 and fig. 4.)

On maltose the larvae likewise outlived decidedly the larvae that were starved; e. g., lot 41, on 35 per cent maltose, lived more than five times as long as lot 43, on water, and lot 88, on 35 per cent maltose, lived more than three times as long as its water check, lot 93. (Tables 10 and 11 and fig. 5.)

TABLE 7.—Average length of life for lots of larvae fed respectively on various concentrations of galactose and on water

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	Hours
94	5 per cent galactose.....	20	19	6.8±0.19
95	15 per cent galactose.....	20	18	8.6±1.86
96	25 per cent galactose.....	20	19	11.38±.35
97	35 per cent galactose.....	20	20	13.51±1.26
98	50 per cent galactose.....	20	17	16.02±.91
99	Water.....	20	17	8.40±.44

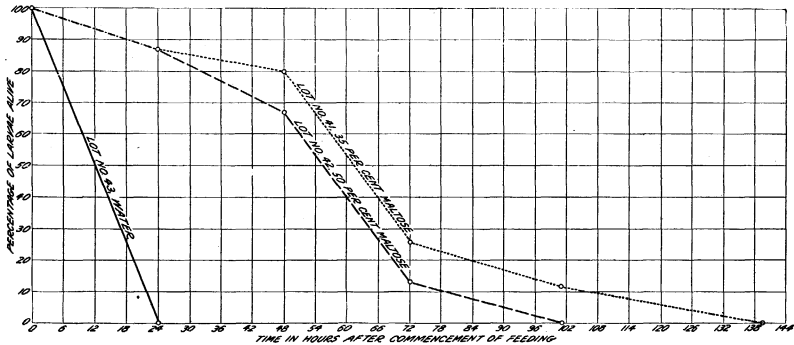


FIG. 5.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of maltose in certain concentrations with water, and of one lot supplied with water only

TABLE 8.—Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on sucrose in specified concentrations with water, and lots supplied with water only, as checks^a

Lot 35 (25 per cent)		Lot 36 (35 per cent)		Lot 37 ^b (water)		Lot 83 (35 per cent)		Lot 93 ^c (water)	
Time	Surviv- ing	Time	Surviv- ing	Time	Surviv- ing	Time	Surviv- ing	Time	Surviv- ing
Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent
5.5	100	5.5	100	5.5	100	6.0	95	3.5	95
23.0	90	22.5	90	22.5	30	11.5	95	10.0	35
29.5	90	29.0	90	29.0	30	18.0	95	16.0	0
47.0	60	46.5	80	46.5	0	24.0	90		
53.5	60	53.5	70			30.0	90		
70.5	60	70.0	70			35.5	90		
77.0	50	77.0	70			42.0	90		
94.5	40	94.0	40			48.0	75		
102.0	30	101.5	40			54.0	55		
118.5	10	118.0	40			59.5	30		
129.5	0	129.0	30			66.0	20		
		166.0	10			72.0	10		
		190.0	0			78.0	10		
						84.0	5		
						90.0	0		

^a Data presented graphically in fig. 4.
^b Check on lots 35 and 36.
^c Check on lot 83.

TABLE 9.—Average length of life for lots of larvae fed respectively on solutions of sucrose and on water

Lot No.	Food	Number of larvae	Weight of each	Length of life
			<i>Mgm.</i>	<i>Hours</i>
34	15 per cent sucrose.....	10	42	45.20± 3.45
35	25 per cent sucrose.....	10	44	73.12± 8.15
36	35 per cent sucrose.....	10	41	95.50±11.30
37	Water (check on lots 34, 35, 36).....	10	45	21.27± 2.48
80	5 per cent sucrose.....	20	16	11.80± .62
81	15 per cent sucrose.....	20	17	17.98± 1.18
82	25 per cent sucrose.....	20	18	32.90± 2.95
83	35 per cent sucrose.....	20	18	54.00± 2.82
84	50 per cent sucrose.....	20	19	59.66± 4.23
93	Water (check on lots 80, 81, 82, 83, 84).....	20	18	8.68± .15
125	75 per cent sucrose.....	20	17	68.85± 7.11
122	100 per cent sucrose.....	20	14	52.28± 3.47
129	Water (check on lots 125, 122).....	20	17	4.61± .23

TABLE 10.—Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on maltose in specified concentrations with water, and a lot supplied with water only, as a check^a

Lot 41 (35 per cent maltose)		Lot 42 (50 per cent maltose)		Lot 43 (water)	
Time	Surviving	Time	Surviving	Time	Surviving
<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>
24.0	87	24.0	87	24	0
48.5	80	48.5	67	-----	-----
72.0	27	72.0	13	-----	-----
101.0	13	101.0	0	-----	-----
139.5	0	-----	-----	-----	-----

^a Data presented graphically in fig. 5.

TABLE 11.—Average length of life for lots of larvae fed respectively on solutions of maltose and on water

Lot No.	Food	Number of larvae	Weight of each	Length of life
			<i>Mgm.</i>	<i>Hours</i>
38	5 per cent maltose.....	15	18	15.86±1.16
39	15 per cent maltose.....	15	24	60.70±5.13
40	25 per cent maltose.....	15	19	46.95±2.63
41	35 per cent maltose.....	15	22	63.71±4.78
42	50 per cent maltose.....	15	21	52.51±3.92
43	Water (check on lots 38, 39, 40, 41, 42).....	15	23	(12 (between 0 and 24)
85	5 per cent maltose.....	20	19	11.20± .46
86	15 per cent maltose.....	20	18	18.40±1.31
87	25 per cent maltose.....	18	19	23.00±1.47
88	35 per cent maltose.....	18	17	28.06±2.90
89	50 per cent maltose.....	20	14	13.87±1.24
93	Water (check on lots 85, 86, 87, 88, 89).....	20	18	8.68± .15

The lots fed on lactose were run in two series. In the first series the greatest age was reached by lot 51 (Tables 12 and 13), but since the first examination was not made until 20 hours after the beginning of the experiment, and all the larvae in water were dead, as shown in Figure 6, A, it was impossible to give the length of life of the water check of the first series, lot 52, except as between 0 and 20 hours. For this reason another series of lots was run with examinations at

6-hour intervals. (Fig. 6, B.) In this series all lots showed a longer life on lactose than on water, lot 92, on a saturated solution of lactose,

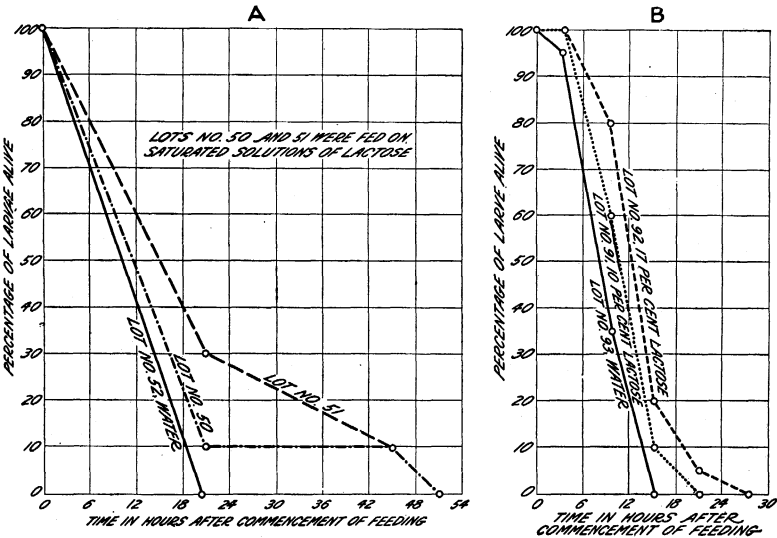


FIG. 6.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of lactose in certain concentrations with water, and of lots supplied with water only, as checks. A, two lots, fed on saturated solutions of lactose, and one, supplied with water only; all the larvae of this lot were found to have died in the first interval of 20 hours. B, two lots, fed on lactose in different concentrations, and one, supplied with water only

living 13.18 ± 0.66 hours, as compared with 8.68 ± 0.15 hours for the water check, lot 93, a ratio of about $1\frac{1}{2}$ to 1. This seems to indicate that the larvae are able to digest lactose.

TABLE 12.—Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on lactose in specified concentrations with water, and lots supplied with water only, as checks^a

Lot 50 (saturated solution)		Lot 51 (saturated solution)		Lot 52 ^b (water)		Lot 91 (10 per cent)		Lot 92 (17 per cent)		Lot 93 ^c (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent
20.5	10	21.0	30	20.5	0	4.0	100	3.5	100	3.5	95
44.5	10	44.0	10	-----	-----	10.5	60	10.0	80	10.0	35
51.0	0	51.0	0	-----	-----	16.5	10	16.0	20	16.0	0
-----	-----	-----	-----	-----	-----	22.0	0	21.5	5	-----	-----
-----	-----	-----	-----	-----	-----	-----	-----	27.5	0	-----	-----

^a Data presented graphically in fig. 6.
^b Check on lots 50 and 51.
^c Check on lots 91 and 92.

TABLE 13.—Average length of life for lots of larvae fed respectively on solutions of lactose and on water

Lot No.	Food	Number of larvae	Weight of each	Length of life
				<i>Mgm.</i> <i>Hours</i>
48	5 per cent lactose.....	10	17	{ 10 (between 0 and 20). 12.97±1.12 13.95±1.87 18.60±3.19
49	Saturated solution lactose.....	10	18	
50	do.....	10	20	
51	do.....	10	22	{ 10 (between 0 and 20). 11.62±.64 11.57±.59 13.18±.66 8.68±.15
52	Water (check on lots 48, 49, 50, 51).....	9	20	
90	5 per cent lactose.....	20	15	
91	10 per cent lactose.....	20	16	
92	17 per cent lactose.....	20	15	
93	Water (check on lots 90, 91, 92).....	20	18	

The larvae fed on trehalose lived longer on all concentrations tested than they did on water. A comparison of the lengths of life of lot 112 and lot 121 (Tables 14 and 15 and fig. 7) gives a ratio of approximately 3 to 1. It appears, then, that the larvae, like the

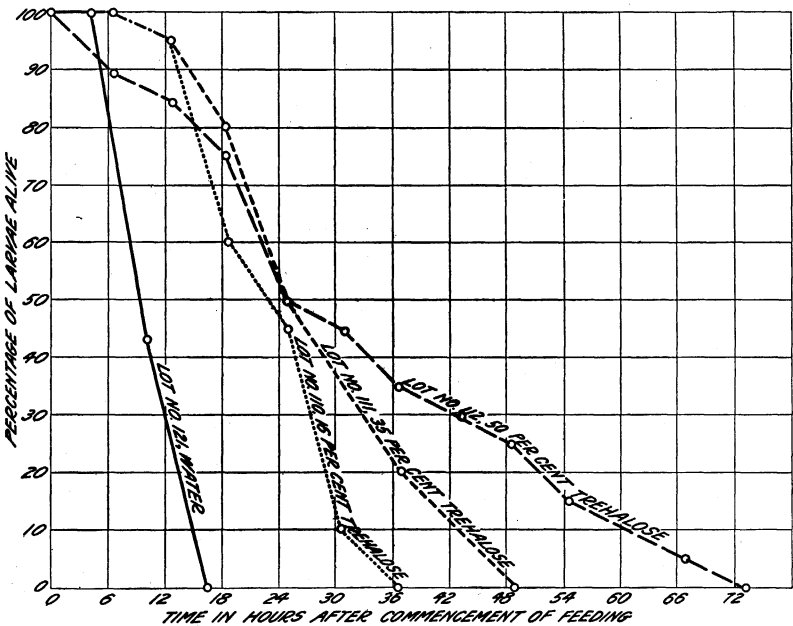


FIG. 7.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of trehalose in certain concentrations with water, and of one lot supplied with water only

adults, can digest this rare sugar with considerable readiness, although it has probably never been a constituent of their diet.

It was found, then, that bee larvae can digest all the disaccharides tested, and that in ability to sustain life these appear to rank in the order sucrose, maltose, trehalose, and lactose.

TABLE 14.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on trehalose in specified concentrations with water, and one lot supplied with water only, as a check*^a

Lot 110 (16 per cent)		Lot 111 (35 per cent)		Lot 112 (50 per cent)		Lot 121 (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>
7.0	100	7.0	100	7.0	90	4.5	100
13.5	95	13.5	95	13.5	85	10.5	43
19.5	60	19.5	80	19.5	75	17.0	0
25.5	45	25.5	50	25.5	50	-----	-----
31.0	10	31.0	35	31.0	45	-----	-----
37.0	0	37.0	20	37.0	35	-----	-----
-----	-----	43.5	10	43.5	30	-----	-----
-----	-----	49.5	0	49.5	25	-----	-----
-----	-----	-----	-----	55.5	15	-----	-----
-----	-----	-----	-----	67.0	5	-----	-----
-----	-----	-----	-----	73.5	0	-----	-----

^a Data presented graphically in fig. 7.TABLE 15.—*Average length of life for lots of larvae fed respectively on solutions of trehalose and on water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			<i>Mgm.</i>	<i>Hours</i>
110	16 per cent trehalose.....	20	13	22.95±1.01
111	35 per cent trehalose.....	20	13	27.75±1.50
112	50 per cent trehalose.....	20	15	31.17±2.89
121	Water.....	23	13	10.21±.43

TRISACCHARIDES

The only trisaccharide used in these experiments was melezitose, a sugar occurring in the sap of certain plants and in honeydew. The data indicate clearly that honeybee larvae are able to use melezitose as food, the length of life for lot 104, on 50 per cent melezitose, being about four and one-third times that of lot 109, on water. (Tables 16 and 17 and fig. 8.)

TABLE 16.—*Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on melezitose in specified concentrations with water, and one lot supplied with water only, as a check*^a

Lot 102 (25 per cent)		Lot 103 (35 per cent)		Lot 104 (50 per cent)		Lot 109 (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>
6.0	95	6.0	100	6.0	100	6.0	94
12.5	90	12.5	95	12.5	90	12.0	0
18.5	60	18.5	60	18.5	70	-----	-----
24.5	45	24.5	50	24.5	65	-----	-----
30.5	20	30.5	35	30.5	55	-----	-----
36.5	10	36.5	35	36.5	45	-----	-----
42.5	10	42.5	20	42.5	35	-----	-----
48.5	0	48.5	15	48.5	30	-----	-----
-----	-----	62.5	0	62.5	20	-----	-----
-----	-----	-----	-----	67.5	20	-----	-----
-----	-----	-----	-----	77.5	0	-----	-----

^a Data presented graphically in fig. 8.

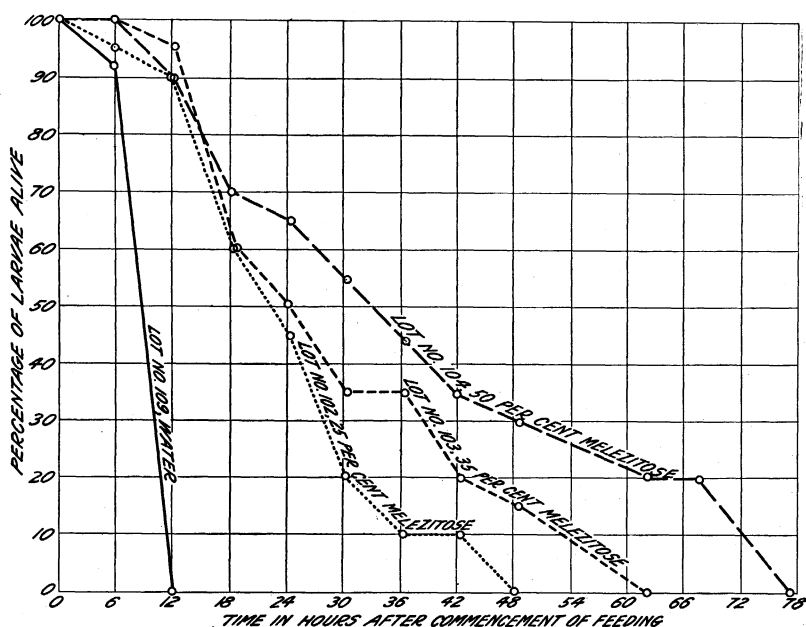


FIG. 8.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on solutions of melezitose in certain concentrations with water, and of one lot supplied with water only

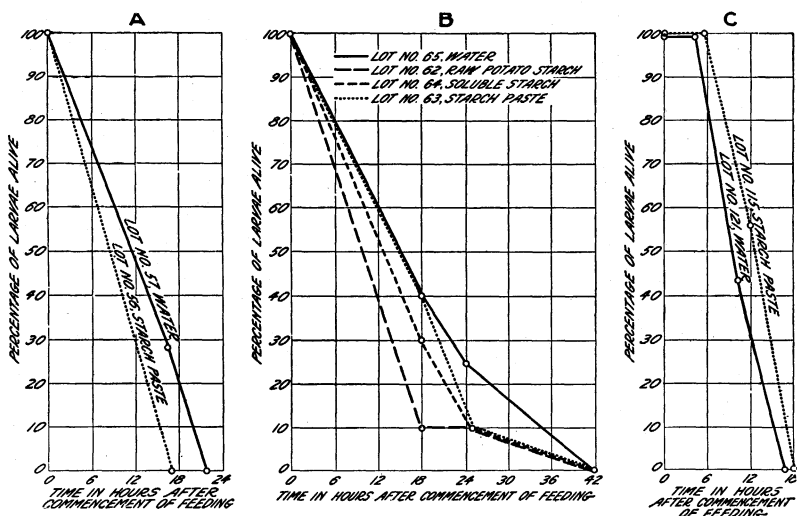


FIG. 9.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding, fed on combinations of starch and water, and others supplied with water only as checks. A, lot fed on starch paste, and lot supplied with water; B, three lots, fed respectively on raw potato starch, starch paste, and soluble starch, and one lot supplied with water only; C, one lot fed on starch paste, and one supplied with water only

TABLE 17.—Average length of life for lots of larvae fed respectively on solutions of melezitose and on water

Lot No.	Food	Number of larvae	Weight of each	Length of life
			Mgm.	Hours
100	5 per cent melezitose.....	20	11	9.25±0.28
101	15 per cent melezitose.....	20	11	16.30±1.05
102	25 per cent melezitose.....	20	11	23.26±2.56
103	35 per cent melezitose.....	20	11	28.58±2.24
104	50 per cent melezitose.....	20	11	37.47±3.31
109	Water.....	16	11	8.62±.24

POLYSACCHARIDES

The polysaccharides used in these experiments were dextrin, glycogen, and starch. The data obtained for these three carbohydrates show that of the five lots fed on starch only one (lot 115) lived longer than the corresponding water check, and even that one less than two hours longer. (Tables 18 and 20 and figs. 9 and 10.) There was no significant difference between the length of life of the lot fed on glycogen (lot 114) and its water check, lot 121. (Tables 18 and 20 and fig. 10.) The lot fed on dextrin (113), however, lived nearly three times as long as the corresponding lot (121) fed on water alone. (Tables 19 and 20 and fig. 10.)

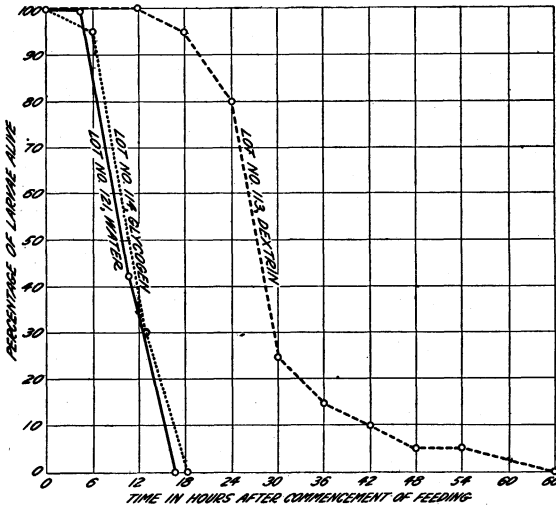


FIG. 10.—Percentages of survivors of three lots of honeybee larvae, at intervals after the commencement of feeding; one supplied with water only, as a check, and the others fed on solutions of dextrin and glycogen, respectively

TABLE 18.—Observations to ascertain the relative duration of life of representative lots of honeybee larvae fed on combinations of starch and water, and lots supplied with water only, as checks ^a

Lot 56 (starch paste)		Lot 57 ^b (water)		Lot 62 (raw starch)		Lot 63 (starch paste)		Lot 64 (soluble starch)		Lot 65 ^c (water)		Lot 115 (starch paste)		Lot 121 ^d (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hrs.	P. ct.	Hrs.	P. ct.	Hrs.	P. ct.	Hrs.	P. ct.	Hrs.	P. ct.	Hrs.	P. ct.	Hrs.	P. ct.	Hrs.	P. ct.
17	0	16.5	29	18.0	10	18.0	40	18.0	30	18.0	40	5.5	100	4.5	100
-----	-----	22.0	0	24.5	10	24.5	10	24.5	10	24.5	25	12.0	55	10.5	43
-----	-----	-----	-----	41.5	0	41.5	0	41.5	0	41.5	0	18.0	0	17.0	0

^a Data presented graphically in fig. 9.

^b Check on lot 56.

^c Check on lots 62, 63, and 64.

^d Check on lot 115.

The results indicate that the larvae are able to utilize dextrin, but neither glycogen nor starch.

It should be added here that an examination of larvae which had been floating on a starch mixture for a few hours showed countless grains of starch in the intestine, and therefore the failure to gain nourishment from starch was not due to a failure to take the starch into the alimentary tract.

TABLE 19.—*Observations to ascertain the relative duration of life of three lots of honeybee larvae, one supplied with water only, as a check, and the others fed on solutions of dextrin and glycogen, respectively*^a

Lot 113 (30 per cent dextrin)		Lot 114 (50 per cent glycogen)		Lot 121 (water)	
Time	Surviving	Time	Surviving	Time	Surviving
<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>
6.0	100	6.0	95	4.5	100
12.5	100	12.5	30	10.5	43
18.5	95	18.5	0	17.0	0
24.5	80				
30.0	25				
36.0	15				
42.5	10				
48.5	5				
54.5	5				
66.5	0				

^a Data presented graphically in fig. 10.

TABLE 20.—*Average length of life for lots of larvae fed respectively on starch, dextrin, glycogen, and water*

Lot No.	Food	Number of larvae	Weight of each	Length of life
			<i>Mgm.</i>	<i>Hours</i>
56	Starch paste.....	10	23	{ 8.5 (between 0 and 17)
57	Water (check on lot 56).....	7	23	
62	Raw starch.....	20	43	11.39±1.54
63	Starch paste.....	20	42	11.40±.85
64	Soluble starch.....	20	46	15.07±1.41
65	Water (check on lots 62, 63, 64).....	20	46	13.85±1.32
115	Starch paste.....	20	42	16.83±1.91
114	50 per cent glycogen.....	20	15	12.18±.46
113	30 per cent dextrin.....	20	12	10.81±.50
121	Water (check on lots 115, 114, 113).....	20	15	29.55±1.24
		23	13	10.21±.43

HONEYS

A study of the ability of honeybee larvae to utilize carbohydrates as food would, of course, be incomplete without an investigation of their relative ability to use honey, which is the main carbohydrate constituent of their normal diet. Bees gather nectar from many sources, and from the different nectars make honeys varying considerably in physical and chemical characteristics. Since, as is well known among beekeepers, these differ in value as food for adult bees, it is probable that they also differ in value as food for the brood.

In order to investigate this question, several lots of larvae were fed on honeys differing as widely as possible in source, color, odor, taste, dextrin content, dextrose-levulose ratio, tannin content, sucrose con-

tent, and the like. These honeys were from sage, white clover, alfalfa, tulip tree, aster, goldenrod, buckwheat, wild buckwheat, mountain laurel, locust, and basswood clover, the latter consisting of three parts of basswood to one of clover. At the same time, for check and comparison, lots were also fed on distilled water and on 75 per cent cane sugar.

The average length of life of the lots fed on the various honeys ranges from 59.82 ± 3.89 hours on basswood clover to 12.39 ± 0.84 hours on locust. (Table 22.) All the honeys tested may be arranged in the following order from greatest to least length of life: Basswood clover, white clover, tulip tree, alfalfa, buckwheat, mountain laurel (poisonous to humans), sage, wild buckwheat, aster, goldenrod, and locust. The average for the 11 honeys tried is 38.93 hours (probable error disregarded), which is essentially the same as for lot 138, fed on 75 per cent cane sugar, and is approximately three and one-half times as long as for lot 139, fed on water alone. (Tables 21 and 22 and fig. 11.)

TABLE 21.—Observations to ascertain the relative duration of life of representative lots of honeybee larvae; one fed on 75 per cent solution of cane sugar, one on locust honey, one on a mixture of 75 per cent of basswood honey and 25 per cent of clover honey, and one supplied with water only as a check ^a

Lot 140 (75 per cent basswood honey, 25 per cent clover honey)		Lot 142 (locust honey)		Lot 138 (75 per cent cane sugar)		Lot 139 (water)	
Time	Surviving	Time	Surviving	Time	Surviving	Time	Surviving
Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent
5.0	100	4.5	92	5.0	96	5.0	100
16.5	96	16.0	16	16.5	72	16.5	0
23.0	96	22.5	12	23.0	72	-----	-----
28.5	96	28.0	8	28.5	68	-----	-----
33.5	84	33.0	4	33.5	68	-----	-----
41.0	68	40.5	0	41.0	44	-----	-----
47.0	64	-----	-----	47.0	32	-----	-----
53.0	48	-----	-----	53.0	28	-----	-----
65.0	44	-----	-----	65.0	20	-----	-----
71.0	32	-----	-----	71.0	16	-----	-----
77.0	28	-----	-----	77.0	8	-----	-----
89.0	20	-----	-----	89.0	4	-----	-----
95.0	12	-----	-----	95.0	4	-----	-----
101.0	8	-----	-----	101.0	4	-----	-----
113.0	0	-----	-----	113.0	0	-----	-----

^a Data presented graphically in fig. 11.

These data show no correlation between length of life of the larvae and any known physical or chemical characteristics of the honey. For example, the length of life on the light honeys, white clover, alfalfa, sage, locust, and basswood clover, averaged 41+ hours, which is not significantly different from the average of 39+ hours on the darker honeys, tulip tree, buckwheat, wild buckwheat, and aster; the tulip-tree honey, with high dextrin content,⁵ sustained life as long as sage, clover, or alfalfa, with low dextrin content; tulip tree and buckwheat, strong in flavor and odor and giving strong tannin reactions, had about the same effect as the milder

⁵ For chemical analysis of honeys, see Browne (5).

sage and basswood clover, having very little tannin; sage, with a high dextrose-levulose ratio, was practically equal in life-sustaining value to alfalfa, with low dextrose-levulose ratio.

TABLE 22.—Average length of life for lots of larvae fed respectively on various honeys, cane sugar, and water

Lot No.	Food	Number of larvae	Length of life
			Hours
130	Sage honey.....	25	39.44±3.09
131	White clover honey.....	25	51.23±3.69
132	Alfalfa honey.....	25	45.39±4.13
133	Tulip-tree honey.....	25	50.16±3.25
134	Aster honey.....	25	24.79±2.09
135	Goldenrod honey.....	25	18.72±1.69
136	Buckwheat honey.....	25	44.57±2.40
137	Wild buckwheat honey.....	25	38.45±2.64
140	Basswood honey, 75 per cent; clover honey, 25 per cent.....	25	59.82±3.89
141	Mountain laurel honey (poison).....	25	43.30±1.74
142	Locust honey.....	25	12.39±.84
	Average of all honeys.....		38.93
138	75 per cent cane sugar.....	25	40.86±3.53
139	Water.....	25	10.70

* The larvae were not weighed, but all lots are comparable as to size and age.

It is a fact, however, that not all honeys were of equal value in sustaining life. This fact may have been due to an unknown characteristic of the honeys, to an uncontrolled factor in the experiment, or to a difference in the larvae themselves.

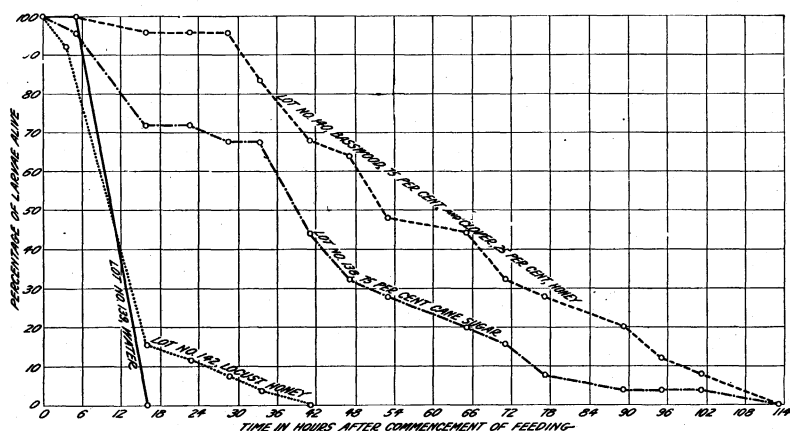


FIG. 11.—Percentages of survivors of lots of honeybee larvae, at intervals after the commencement of feeding; one fed on a 75 per cent solution of cane sugar, one on locust honey, one on a mixture of 75 per cent of basswood honey and 25 per cent of clover honey, and one supplied with water only.

REVIEW AND COMPARISON

In the preceding pages have been presented in detail the results obtained in experiments on honeybee larvae fed on solutions of the monosaccharides, dextrose, levulose, and galactose; of the disaccharides, sucrose, maltose, lactose, and trehalose; of the trisaccharide, melezitose; and of the polysaccharides, dextrin, starch, and

glycogen; and on various honeys. A comparison of the life-sustaining value of these various carbohydrates can now readily be made by first computing a ratio for each carbohydrate and then comparing the ratios. The ratio in each case is found by dividing the length of life of the optimum lot in each series by the length of life of its particular water check. In case more than one series of lots were run for a single carbohydrate, an average is taken of the ratios of all the series for that carbohydrate. (Table 23.) A ratio of one, or less than one, means, of course, that the length of life on that carbohydrate was equal to or less than the length of life on water, and indicates no food value at all.

TABLE 23.—Comparison of various carbohydrates as to their value in sustaining the life of honeybee larvae. All carbohydrates tried appear to have food value except starch and glycogen (probable error disregarded)

Name	Carbohydrate (=C)		Starvation check (water) (=S)		Ratio (C/S)	Average ratio (C/S)
	Lot No.	Length of life	Lot No.	Length of life		
Sucrose	36	95.50	37	21.27	4.5	-----
Do	84	59.66	93	8.68	6.9	-----
Do	125	68.85	129	4.61	14.9	-----
Do	138	40.86	139	10.70	3.8	7.5
Levulose	79	47.56	73	10.10	4.7	4.7
Maltose	41	63.71	43	12.00	5.3	-----
Do	88	28.06	93	8.68	3.2	4.3
Melezitose	104	37.47	109	8.62	4.3	4.3
Dextrose	75	39.90	73	10.10	4.0	4.0
Honeys	(a)	38.93	139	10.70	3.6	3.6
Trehalose	112	31.17	121	10.21	3.1	3.1
Dextrin	113	29.55	121	10.21	2.9	2.9
Galactose	98	16.02	99	8.40	1.9	1.9
Lactose	51	18.60	52	10.00	1.9	-----
Do	92	13.18	93	8.68	1.5	1.7
Starch	56	8.50	47	11.39	.7	-----
Do	63	15.07	65	16.83	.9	-----
Do	115	12.18	121	10.21	1.2	.9
Glycogen	114	10.81	121	10.21	1.1	1.1

^a Average.

These data show (Table 23) that sucrose, with a ratio of 7.5, had the greatest life-sustaining value, and that following this came, in order, levulose (4.7), maltose (4.3), melezitose (4.3), dextrose (4.0), honeys (3.6), trehalose (3.1), dextrin (2.9), galactose (1.9), lactose (1.7), glycogen (1.1), and starch (0.9). Glycogen and starch, therefore, had no more food value than distilled water.

ENZYMES

Practically all work done on digestion in insects has been a study of the various enzymes produced in the insect, either in the body as a whole or in some particular part of it. If the results obtained by feeding are to be compared with results obtained by investigating the enzymes, it follows that both must be reduced to common terms.

Our knowledge of digestion in general indicates that an organism which is able to get nourishment from a carbohydrate other than a monosaccharide must first change this carbohydrate to a monosaccharide. Likewise, our knowledge of enzymes indicates that a specific enzyme is necessary for the hydrolysis of each complex carbohydrate

to a simpler form. It therefore seems clear that if an insect is able to obtain nourishment from a complex carbohydrate it must produce the specific enzyme necessary to hydrolyze this carbohydrate, and it must secrete this enzyme at a place where it may be in contact with the food.

To state the results of these experiments in terms of enzymes, it seems clear that the honeybee larva has available for use in digestion, the following enzymes: Invertase, maltase, melezitase, trehalase, dextrinase, and lactase, but does not have available any diastase.

The fact that there is no diastase available for use in digestion was quite unexpected, in view of the apparent widespread distribution of diastase among insects and its presence even in the adult honeybee, as reported by Erlenmeyer, Axenfeld, Petersen, and Pavlovsky and Zarin. (Table 1.) But is it not possible for diastase to be produced in an insect and still not be secreted into the intestine?

In order to obtain direct evidence concerning this question a number of larvae were punctured and their coelomic fluid (blood) collected. A few cubic centimeters of this fluid were added to a thin starch paste and incubated overnight at a temperature of 37.5 °C. The next morning the starch had disappeared and the solution gave a distinct reaction to Fehling's test for sugar, thus showing clearly that diastase is present in the coelomic fluid. If, now, it be recalled that, as before mentioned, the larvae take starch into their alimentary tract, but receive no nourishment from it, it seems evident that although diastase is present in the larva as a whole, it is not present in the lumen of its digestive tract. This conclusion is essentially the same as that of Phillips (17) for the adult honeybee.

At the same time, tests were made to ascertain whether or not invertase, maltase, and lactase were present in the coelomic fluid. Coelomic fluid was added to solutions of pure sucrose, maltose, and lactose, and the hydrogen-ion concentration was adjusted to P_H 6.8. Then the specific rotation of the solution was ascertained by means of the polariscope, after which the solution was covered with toluol and allowed to stand for 24 hours. The polariscope reading was then again taken but in no case was any significant change noted. Such negative results give an indication, although by no means a proof, that neither invertase, maltase, nor lactase is present in the coelomic fluid of the larva, although from feeding experiments these enzymes are shown to be available for use in digestion.

SUMMARY AND CONCLUSIONS

Honeybee larvae may be removed from the comb and kept alive under artificial conditions such as are described under the head, "Material and method."

They are able to utilize as food the following carbohydrates, named in the order of their apparent food value: Sucrose, levulose, maltose, melezitose, dextrose, trehalose, dextrin, galactose, and lactose (the last very slightly). This fact indicates that the larva has available the digestive enzymes invertase, maltase, melezitase, trehalase, dextrinase, and lactase. Honeybee larvae apparently differ from adult honeybees, then, in their ability to use dextrin and galactose (according to Phillips) and lactose (according to both Phillips and Pavlovsky and Zarin). Lactase, has, however, been

found in the larvae of *Euproctis chrysorrhoea*, *Bombyx mori*, *Hyponomeuta* sp., and *Tenebrio molitor*. (Table 1.)

Two carbohydrates which honeybee larvae are not able to use as food are starch and glycogen. The enzyme diastase is therefore not present in the lumen of the gut. But the enzyme diastase is present in the coelomic fluid of the larvae, as shown by the ability of the coelomic fluid to digest starch. In this respect, according to Phillips, the larvae are like the adult honeybees; according to all other investigators they are unlike the adults. They are also apparently unlike almost every other insect which has been investigated.

Larvae are like adults in their ability to metabolize two rare sugars, melezitose and trehalose. Their ability to use trehalose is especially remarkable in view of the fact that this sugar probably never occurs in their normal diet.

Under the conditions of these experiments honeys show a life-sustaining value slightly less than sucrose, and approximately equal to levulose, maltose, melezitose, and dextrose. The various honeys used ranked in the following order from greatest to least life-sustaining value: Basswood clover, white clover, tulip tree, alfalfa, buckwheat, mountain laurel, sage, wild buckwheat, aster, goldenrod, and locust; the reason for this order of values is not clear, since it is not to be explained on the basis of any known physical or chemical characteristic of the honeys.

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