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Total Carbohydrate and Glycogen Content of the Developing Honeybee.*†

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It has been reported that the concentration of total reducing substances, calculated as glucose, increases during the larval stage of the queen and worker honeybee (*Apis mellifera* L.) and decreases during pupation.[1] A further study presented here deals with changes in the total carbohydrate, glycogen, and free reducing sugar that accompany the development of the worker honeybee.

A review of some of the earlier work shows that investigators have been able to establish certain significant facts concerning the carbohydrate metabolism of the honeybee. Straus[2] reported that toward the end of the larval period of the worker honeybee more than 30% of the dry weight was glycogen, whereas Parhon[3] found the adult to contain but 0.14-0.21%. Straus[2] was able to detect little, if any, reducing sugar in the tissues of the developing worker bee; however, Sturtevant[4] found reducing sugar in 60% alcohol extracts of bee larvae. Moreover, both glucose and glycogen were found in the hemolymph of the prepupal honeybee by Ronzoni and Bishop.[5]

Experimental. Total carbohydrate, glycogen, and free reducing sugar were determined at 24-hour intervals during the life cycle of the honeybee. The methods of rearing insects and preparation of tissue for analysis have been described.[6] Worker honeybees complete their development in 18 days after hatching; feeding occurs during the first 5 days of larval life; pupation takes place on the 8th day of larval life; and the imago emerges 10 days later.

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1 Melampy, R. M., Willis, E. R., and McGregor, S. E., *Physiol. Zool.*, 1940, **13**, 283.

2 Straus, J., *Z. Biol.*, 1911, **56**, 347.

3 Parhon, M., *Ann. Sci. Nat., Zool.*, 1909, **9**, 1.

4 Sturtevant, A. P., *J. Agr. Res.*, 1924, **28**, 129.

5 Ronzoni, E., and Bishop, G. H., *Trans. IV Inter. Cong. Ent.*, 1929, 361.

6 Melampy, R. M., and Willis, E. R., *Physiol. Zool.*, 1939, **12**, 302.

For the first 2 days after hatching the larvae are fed royal jelly, a secretion of the pharyngeal glands of the workers. During the third day the diet of the worker larvae is altered to include pollen and honey.

Total Carbohydrate. Total carbohydrate includes free sugar of the tissues, unassimilated carbohydrates in the alimentary canal, glucose produced by hydrolysis of the glycogen, and other carbohydrates convertible by acid hydrolysis into reducing sugar. In determining the total carbohydrate approximately 1 g of macerated tissue was placed in a 50-cc centrifuge tube, and 10 cc of 1.2 N sulfuric acid was added. The tube was closed with a rubber stopper that was provided with a 2-foot glass tube to serve as a reflux condenser. The tubes and their contents were heated for 3 hours in a boiling-water bath. When the digests were cool, they were neutralized with a saturated aqueous solution of barium hydroxide (phenolphthalein being used as an indicator), diluted to 50 or 100 cc and filtered. The solution was analyzed for its reducing power before and after yeast fermentation by the dinitrosalicylic acid method of Sumner[7] or the iodometric titration of reduced copper.[8]

Glycogen. The glycogen determinations were made by the Good, Kramer, and Somogyi modification of the Pflüger method.[9] The glycogen was hydrolyzed by heating for 2.5 to 3 hours with 5 cc of 1 N sulfuric acid. The acid hydrolyzates were neutralized with a saturated aqueous solution of barium hydroxide, phenolphthalein being used as an indicator. The solution was analyzed for its reducing power before and after yeast fermentation by the method of Sumner[7] or Somogyi.[8]

Free Reducing Sugar. The procedure of Steiner, Urban, and West[10] was used to prepare tissue filtrates for the determination of free reducing sugar in the body tissues of the developing honeybee. The reducing power of the filtrates before and after yeast fermentation was determined by Somogyi's method.[8]

Yeast Fermentation. Benedict's method[11] was used for preparing yeast for the fermentation of filtrates. About half a yeast cake was thoroughly mixed with 40 cc of distilled water in a 50-cc centrifuge tube, and the mixture was centrifuged for 3 minutes. After 4 such washings, the yeast was suspended in 50 cc of water,

7 Sumner, J. B., *J. Biol. Chem.*, 1925, **65**, 393.

8 Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

9 Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

10 Steiner, A., Urban, F., and West, E. S., *J. Biol. Chem.*, 1932, **98**, 289.

11 Benedict, S. R., *J. Biol. Chem.*, 1928, **76**, 457.

and 10 cc aliquots were transferred to 15 cc centrifuge tubes. After being centrifuged, the tubes were inverted on sheets of filter paper to drain for a few seconds, and the adhering film of moisture on the inside of the tube was removed by wiping with filter paper. The tissue filtrate was added and mixed with the yeast. Fermentation was allowed to proceed for approximately 30 minutes, after which the mixture was centrifuged and the reducing power of the filtrate determined.

Results. The results from the analyses for total carbohydrate, glycogen, and free reducing sugar are presented in Table I. Each value represents the average of 6 determinations.

Discussion. Table I shows that during the larval stage the total carbohydrate and glycogen increase. The maximum storage of both of these materials occurs between the fifth and sixth days, which is approximately the end of the larval feeding period and the time the cell is sealed. The total carbohydrate at this time is 100 mg and the glycogen 68 mg per gram of tissue; however, 12 days later the total carbohydrate has decreased to 9 mg and the glycogen to 0.9 mg per gram. If it may be assumed that the daily rate of utilization is constant, the total carbohydrate decreases approximately at the rate of 8 mg and the glycogen at the rate of 6 mg per gram per day.

It is of interest that the synthesis and utilization of total carbohydrate and glycogen parallel each other throughout the life cycle. However, the glucose due to hydrolysis of glycogen varies at different stages of development; for example, at the 3-4-day stage it comprises 42% of the total carbohydrate, at the 5-6-day stage

TABLE I.
Total Carbohydrate, Glycogen, and Free Reducing Sugar of the Developing Worker Honeybee.

Age days	Stage	Expressed as mg of glucose per g of tissue		
		Total carbohydrate	Glycogen	Free reducing sugar
3-4	Larval	24	10	5
4-5	"	77	50	3
5-6	"	100	68	1
6-7	"	89	66	0.3
7-8	"	82	65	0.5
8-9	Pupal	75	57	0.8
9-10	"	66	51	0.9
10-11	"	59	47	0.9
11-12	"	55	42	1.0
12-13	"	50	35	1.0
13-14	"	38	25	1.3
14-15	"	31	19	1.5
15-16	"	26	12	1.8
16-17	"	19	10	2.0
17-18	Imagal	9	0.9	1.5

68%, and at the 17-18-day stage 10%. At no time does the free reducing sugar make up the difference existing between the total carbohydrate and glycogen. The greatest difference is 32 mg per gram of tissue at the 5-6-day stage, but at this time the free reducing sugar is only 1.0 mg per gram. The free reducing sugar decreases to 0.3 mg per gram at the 6-7-day stage, rises to 2.0 mg per gram by the 16-17-day stage, and decreases before emergence. It is clearly shown, from the results presented here, that some form of carbohydrate other than glycogen and free reducing sugar must be present. This carbohydrate is convertible, by acid hydrolysis, to reducing sugar. It is either destroyed by the alkali used in the glycogen procedure or not precipitated by the concentration of alcohol employed in the glycogen method.

The rapid synthesis of carbohydrate reserves may be accounted for by the presence of readily assimilable carbohydrate, chiefly glucose and levulose, in the diet of the developing worker honeybee. According to Snodgrass,[12] one of the important functions of the fat body of some insects is glycogen storage. In this respect the function is similar to that of the vertebrate liver. Glycogen prepared from prepupal worker honeybees gave a wine-red color with Lugol's iodine solution. Melampy and Willis[6] found the respiratory quotient of the worker honeybee to be greater than unity during the larval period, thus indicating a synthesis of lipid from carbohydrate at that time. The average respiratory quotient during the pupal period of the worker honeybee was 0.96.

The results presented here are in agreement with the findings of other investigators dealing with the carbohydrate metabolism of the developing honeybee. There is close agreement with the data of Straus[2] as regards glycogen, but, in contrast to his findings and in agreement with those of Sturtevant,[4] this investigation indicates the presence of free reducing sugar during development. The carbohydrate reserves of the developing honeybee are probably utilized in the production of energy and in the synthesis of chitin.

Summary. The changes in the concentration of the total carbohydrate, glycogen, and free reducing sugar have been investigated during the larval and pupal development of the worker honeybee. The following results have been obtained:

1. During larval development there is a marked increase in the total carbohydrate and glycogen.

2. During the pupal period both the total carbohydrate and

[12] Snodgrass, R. E., *Anatomy and Physiology of the Honeybee*, McGraw-Hill, 1925.

glycogen decrease. At the time of emergence a very small amount of carbohydrate reserve remains.

3. In addition to glycogen, there is in the tissues of the immature honeybee a reserve carbohydrate which is convertible, by acid hydrolysis, to reducing sugar.

4. Free reducing sugar is found in the tissues of larval and pupal honeybees.