SUBJECT: Progress Report No. 3--Evaluation of Honey for Selected Biological Properties. Contract No. 12-14-100-2596(73).
Period Covered: May 12, 1959, to November 12, 1959.

OBJECTIVES OF THE PERIOD

1. During this period the yeast growth factor in citrus and clover honey was studied.
2. Preliminary studies were carried out to determine conditions for the rooting experiment.
3. The rat growth study was planned.

EXPERIMENTAL METHODS

The yeast growth property of honey was studied by means of the Hertz assay method for biotin (1). Two modifications were used: first, the samples were sterilized by heating at 100°C. for 5 min. at atmospheric pressure, and sterile conditions were maintained throughout the test; second, inoculation of the sample was made quantitative by adding two drops of yeast suspension (0.003 mg) from a No. 25 hypodermic needle. All samples of diluted honey were filtered through a micro-pore filter to produce a clear sample. All turbidity readings were made on a colorimeter at 660 mμ and recorded as percent transmission.

In determining the optimum conditions for study of the root growth effect of honey chrysanthemum cuttings were at first allowed to stand for 1, 2, 4, or 6 hours in the test solution and were then planted in the sterilized perlite[1]. The honey solutions were made up in water to 5, 10, 20, and 50% (v/v) of honey; a positive control of 20 p.p.m. of alpha-naphthalene-acetic acid and a negative control of 50% sugar solution were applied to cuttings in the same manner. The time of exposure of cuttings to the test solution was based on Oliver's work (2).

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[1] Mineral of iron and carbon

Due to unexpected demands of other projects on available animal house space, it was not possible to commence the study of the rat growth factor as intended for this period. However, this study will be initiated shortly, hence the basal diet and several of the honey diets have been prepared as follows: basal diet composed of casein 18%, cornstarch 62.2%, cornstarch-vitamin mixture 10%, salts 4.0%, DL-methionine 0.6%, DL-tryptophane 0.2%, and refined cottonseed oil 5%. The cornstarch-vitamin mixture contained the following substances in 100 g. of mixture: thiamine 50 mg., riboflavin 50 mg., niacin 100 mg., calcium pantothenate 200 mg., pyridoxine 1.0 mg., folic acid 2.0 mg., vitamin B_{12} 0.2 mg., inositol 1 g., and choline 15 g. Weekly administration by dropper of 2 drops of fortified Haliver oil diluted with corn oil to give 400 I.u. vitamin A, 4 I.u. vitamin D, and 0.04 mg. of 2-methyl-1,4-naphthoquinone and 4.0 mg. of alpha-tocopherol. In the test diets 30% of the ~~cornstarch was replaced with~~ diet will be replacing cornstarch honey and for a control diet 30% of the cornstarch was replaced with a mixture of 83% sucrose and 17% water to give isocaloric diets. Because of space limitations in the animal house we will repeat the following experiment at a later date. Groups of ten rats will be fed the test diets containing one of four honeys, raw or heat-treated, or sucrose as control. Feeding will be ad libitum. At the end of four weeks the carcasses will be analyzed for fat and protein to determine the nature of any weight gain.

RESULTS ACCOMPLISHED

Variable results were obtained with the Hertz biotin assay technique. Initial experiments indicated biotin contamination in one or more of the medium constituents. Considerable work showed calcium pantothenate to be the source of contamination. As shown in Table I considerable variation was obtained in the standard biotin curves, depending on incubation time and other indeterminate

factors, which shows the need for setting up a series of standard biotin concentrations with each test carried out. Also included in Table I are the results of adding a series of sugar solutions (synthetic honey) of increasing concentration to tubes of yeast medium containing no biotin.

Table I. Yeast Growth Values in Presence of Biotin or Sucrose

Run I		Run II			
Biotin, mμg.	% Transmission	Biotin, mμg.	% Transmission	% Sugar	% Transmission
0	98	0	99	1	94.5
0.1	78	0.1	82.5	10	92
0.2	63	0.2	68	20	92.5
0.3	57	0.3	63.5	40	91
0.4	51	0.4	61	80	92
0.5	44.5	0.5	55		
0.6	43.5				
0.7	41				
0.8	39				
0.9	36.5				

Note: Sugar solutions determined simultaneously with Run II.

Addition of sugar to the biotin-deficient medium resulted in slight or no growth of yeast cells. This indicated that growth obtained with two honeys (reported below in Table II) did not result from the sugar content of those honeys but rather from biotin or a biotin-like substance.

Table II. Yeast Growth Values in Presence of Raw Honeys

Honey, %	% Transmission	
	Citrus	Clover
1	57.5	37.5
10	18	25.5
20	16.5	22
40	21.5	26
80	101.5	89.5
100	94	100

Note: All determinations made in duplicate; incubated 24 hours; 5 ml. volumes used.

The data in Table II show that the honeys tested thus far in the raw state both contain a growth-stimulating factor for yeast. It was not possible to convert the percentage transmission values (reciprocal of turbidity) to equivalent amounts of biotin in either of the above experiments because of difficulty with the standard series. In the biotin standard carried along with the citrus honey no growth was obtained, and in that with the clover honey all levels of biotin produced the same growth due to the contamination noted above. Despite the lack of standard values it is obvious that maximum yeast growth occurred at the 20% level for each honey while complete inhibition was obtained at the 100% level. A very rough approximation of the amounts of biotin or biotin-like substance in the two honeys, calculated on the basis of 1% of honey and the Rum I biotin standard, showed about 6 mμg./g. of citrus honey and 17 mμg./g. of clover honey.

Chrysanthemum cuttings were used in the preliminary studies on the effect of honey on rooting. The first experiments were carried out in an open tray at

room temperature (32°C.) and humidity (40%); however, even with frequent watering the cuttings succumbed due to excessive transpiration. A mist chamber was constructed to cover a tray 12" x 18" x 4". Cuttings soaked in the honey or the control solutions for various time intervals from 1 hour to overnight and then planted in perlite in the mist chamber maintained their viability but difficulties developed in the form of a fungal or bacterial rot in the root zone of the cuttings. It is possible that the concentrated honey or sugar solutions dehydrated and weakened the cells in the root zone, making them susceptible to rot. A commercial antibiotic fungicide was ineffective in preventing rot. Shorter times of exposure of the cuttings to the test solutions are being tried as well as sterilization of all materials used. The results of one of the preliminary tests in which chrysanthemum cuttings were soaked in solutions of clover honey, synthetic honey, or naphthaleneacetic acid, or in water, are shown below in Table III. After soaking the cuttings were planted in the mist chamber and after 11 days were examined for rooting.

Table III. Effect of Raw Sweet Clover Honey on Chrysanthemum Rooting

Time of Exposure to Solutions, Hr.	Average number of roots per cutting				
	NA Acid	5% Honey	50% Honey	50% Sugar	Water
1	4	6	*[a]	*[b]	1
4	12	5	*	*	1
6	11	3	*	*	1
18	9	1	*	*	0

[a] 4 roots on 1 cutting

[b] 3 roots on 1 cutting

* rotting occurred

According to the results of this one experiment the optimum exposure time to the naphthaleneacetic acid control was 4 hours; however, soaking in 5% honey for only 1 hour gave better results than did any longer periods. Based on these results work is currently underway in which cuttings are soaked in the test solutions for only a few seconds and then planted. It is hoped in this way to minimize or eliminate the rot problem.

FUTURE EXPERIMENTS

1. The yeast growth and rooting studies will be completed.
2. The animal studies will be initiated during the next period and will be planned for completion by September 1, 1960, or earlier.

LITERATURE CITED

1. Hertz, R. "Modification of the yeast-growth assay method for biotin." Proc. Soc. Exp. Biol. Med. 52, 15 (1943).
2. Oliver, R. W. "Honey as a stimulant to the rooting of cuttings." American Bee Journal 80, 158 (1940).

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