SUBJECT: Progress Report No. 6 - Evaluation of Honey for Selected Biological Properties. Contract No. 12-14-100-2596 (73). Period Covered: May 1, 1960 to September 1, 1960.

OBJECTIVES OF THE PERIOD

1. During this period the yeast growth study was completed.
2. The rat growth study was completed except for the carcass protein analysis.
3. The rooting experiment was partially completed.
4. The guinea pig joint anti-stiffness experiment was initiated.

EXPERIMENTAL METHODS

During the period reported eight honeys, heat-treated and untreated, were tested for the yeast growth property by the Hertz assay method for biotin as outlined in progress report No. 3.

Four more heat-treated and untreated honeys were tested for rat growth effect by the procedure outlined in progress report No. 5. Average weight gain, percent of solids and fat in the carcass, and feed efficiency were determined. Analysis of the carcass protein is underway and will be reported in the next progress report.

The rooting experiment was carried out by dipping the cuttings in the test solutions for 5 seconds (progress report No. 3). Chrysanthemum cuttings were allowed to remain in the mist chamber three to four weeks, while pyracantha cuttings remained six to eight weeks. The number of roots per cutting were determined and compared statistically with cuttings dipped in α-naphthaleneacetic acid solution.

Guinea pigs weighing from 200 to 400 g. were placed on a pelleted basal diet similar to that of Oleson et al. (1). This was composed of ground wheat 20 lb, ground whole yellow corn 15 lb., feeding oat meal 20 lb., wheat bran 5 lb., soy bean oil meal (41% expeller) 20 lb., Linseed oil meal 5 lb., fish meal (60% vacuum) 2 lb., alfalfa leaf meal (dehydrated 20%) 10 lb., bone meal (Steamed) 1 lb., limestone (feeding) 1 lb., NaCl 0.25 lb., Vit. D_3 supplement (3000 I. U. vitamin D_3/g.)0.082 lb., and ascorbic acid 12 g. In addition, each animal received orally approximately 20 mg. of ascorbic three times per week.

The degree of stiffness was determined by the manual procedure of van Wagtendonk and Wulzen (2). In this test the foreleg of the animal is extended posteriorly along the body wall, supported from below by the operator's fingers and held rigid by downward pressure on the olecranon process with the operator's thumb. With the other hand the paw is flexed upward by gentle pressure. Ratings of 1 to 4 for severity of the symptoms were used, 1 being very severe and 4 normal. Three-plus animals will be used in the tests.

RESULTS ACCOMPLISHED

Table I shows results obtained in the yeast growth experiment. The values listed are arbitrarily stated in terms of biotin activity and were determined from a standard growth curve of yeast incubated with increasing levels of biotin of the same time as the honey samples. These were corrected for background turbidity initially present in the honey.

It can not be determined from these data whether there is actually a yeast growth stimulating factor in honey, since the growth stimulation caused by biotin alone has not been determined. However, in most cases honey caused more yeast growth than 1000 mμg. of biotin. This is an extremely large amount of biotin since Hertz (3) has reported that 0.5 mug. of biotin will cause maximum growth stimulation of *Saccharomyces cerevisiae*. The concentrations of 10, 20 and 40% of raw honey showed the most activity, therefore each honey was heat-treated and tested at these concentrations. Heat-treating produced variable effects but generally resulted in a decrease in the growth promoting property, indicating that the "yeast-growth factor" is probably heat labile. The various sugar concentrations (positive control) are not listed in Table I since they showed no growth stimulation as described in project report No. 3, Table I.

Sixty-seven guinea pigs have been receiving the basal diet of Oleson *et al.* (1) for 9 weeks. Only two pigs have developed definite symtoms of joint stiffness, however, most of the animals show definite muscle soreness. Feeding of the basal diet will be continued until the "stiffness syndrome" is fully developed in all of the animals.

Table I. Yeast Growth Study

Honey Conc., %	1	10	20	40	80
			Biotin, mug.[1]		
Citrus	1.3	>1000	>1000	100	0
Treated Citrus	0	>1000	>1000	>1000	0
Sweet Clover (Budge)	7.0	25	20	15	0
Treated Sweet Clover (Budge)	0	2.8	3.3	3.0	0
Tupelo	0.8	>1000	>1000	>1000	0
Treated Tupelo	0	>1000	2.3	1.3	0
Tulip	1.7	>1000	>1000	>1000	0
Treated Tulip	0	>1000	1000	1000	0
Buckwheat	100	>1000	>1000	>1000	0
Treated Buckwheat	0	>1000	2.3	1.4	0
Cotton	4.4	>1000	>1000	>1000	0
Treated Cotton	0	>1000	4.5	2.5	0
Fall Flower	0.3	3.7	2.3	1.2	0
Treated Fall Flower	0	>1000	>1000	1000	0
Alfalfa and Sweet Clover (Powers)	0.6	3.3	4.7	3.1	0
Treated Sweet and Alfalfa Clover (Powers)	0	2	3	1.7	0

[1] Activity arbitrarily expressed in terms of biotin standard curve.

Table II shows the results of the rat growth experiment. All the honeys treated or untreated, except for untreated Tupelo, showed significent increases in average weight gain and feed efficiency at the 5% level of significance. The fat and solids values are not significantly different from the control values.

Table II. Influence of Carbohydrate Source on Rat Growth (Five Weeks)

Source of 25% of Carbohydrate Carin Diet	Average Weight Gained, g.	Solids, %	Carcass Fat %	Feed Efficiency
Cornstarch† (Basal)	73.5* ± 8.54	46.75 ± 9.04	10.75 ± 2.38	22.13* ± 2.21
Sucrose (Control)	85.8 ± 13.56	42.68 ± 1.43	11.10 ± 1.27	27.34 ± 2.62
Tupelo	94.0 ± 7.15	40.64 ± 1.53	9.36 ± 1.81	29.04 ± 2.37
Treated Tupelo	101.3* ± 11.54	40.68 ± .69	9.81 ± 1.18	31.01* ± 2.81
Buckwheat	102.3* ± 7.24	41.48 ± 1.59	10.34 ± 2.06	32.71* ± 3.56
Treated Buckwheat	105.0* ± 7.09	40.81 ± 1.35	9.95 ± 2.06	34.05* ± 3.70
Sweet Clover (Powers)	102.1* ± 14.85	39.70 ± 1.64	9.39 ± 1.84	32.87* ± 3.58
Treated Sweet Clover (Powers)	110.1* ± 8.65	41.17 ± .94	10.84 ± 1.32	33.37* ± 2.68
Fall Flower	105.5* ± 11.64	39.54 ± .76	9.10 ± 1.20	33.05* ± 2.67
Treated Fall Flower	99.4* ± 13.55	38.80 ± 3.80	9.34 ± 1.57	32.23* ± 2.69

* significantly different from control at the 5% significance.

† source of 62% of the carbohydrate in the diet.

The results of the rooting experiment completed to date are shown in Table III. With chrysanthemum cuttings only the 20% cotton showed a significant increase over α-naphthaleneacetic acid. ~~Ten and 20 percent fall flower honey caused significantly less rooting in the case of pyracantha.~~ It is interesting to note that although the honeys did not stimulate greater rooting than did α-naphthaleneacetic acid, they were equal to control in most cases.

Table III. Rooting Experiment With Chrysanthemum Cuttings.[1]

Concentration, %	Tulip	Citrus	Buckwheat	Tupelo	Cotton
			Average No. Roots per Cutting		
5	21.2 ± 7.5	24.4 ± 6.0	23.8 ± 7.3	31.1 ± 8.8	29.0 ± 9.6
10	22.1 ± 5.8	23.8 ± 6.3	23.0 ± 7.2	30.9 ± 9.9	24.8 ± 7.1
20	21.8 ± 9.3	25.3 ± 6.1	28.6 ± 6.6	27.2 ± 10.6	36.4 ± 8.8*
50	24.4 ± 6.8	21.0 ± 5.0	27.0 ± 6.4	26.4 ± 9.0*	29.8 ± 7.2
			Sugar Solution		
50	25.6 ± 12.2	21.0 ± 5.9	27.8 ± 7.8	23.6 ± 11.5*	28.8 ±12.3
			α-Naphthaleneacetic acid		
20 ppm.	26.9 ± 12.6	20.0 ± 5.0[2]	27.6 ± 7.0	33.8 ± 9.8	29.6 ± 7.1

Rooting Experiment With Pyracantha Cuttings
Fall Flower Honey

5%	10%	20%	50%	Sugar Solution 50%	α-Naphthaleneacetic acid
3.2 ± 2.2	2.0 ± 1.9	1.7 ± 1.2	2.8 ± 2.1	3.6 ± 2.3	3.2 ± 2.7

1 20 cuttings per sample.
2 Average roots for 8 cuttings.
* Significantly different at the 5% level compared with the α-naphthaleneacetic acid control.

FUTURE EXPERIMENTS

1. The rooting study will be completed.
2. The protein analysis of the rat carcasses will be completed.
3. The guinea pig anti-stiffness experiment will be continued and possibly completed depending on rapidity of development of deficiency syndrome in animals.
4. The estrogenic study will be started.

LITERATURE CITED

1. Oleson, J. J., Evelyn C. Van Donk, Seymour Bernstein, Louis Dorfman, and Y. Subbarow, Steroids and the stiffness syndrome in guinea pigs, J. Biol. Chem., 171, 1-7 (1947).
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3. Hertz, R., Modification of the yeast growth assay method for biotin, Proc. Soc. Exp. Biol. Med., 52, 15 (1943).