IX. THE VITAMIN CONTENT OF HONEY.

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THE experiments recorded in the literature suggest that honey is not a good source of vitamins. Dutcher [1918] tested strained honey from newly-filled combs, obtained while clover and basswood were in bloom, on pigeons and came to the conclusion that the "water-soluble B" content of the product was negligible. Faber [1920] found that daily doses of 4-8.4 cc. of white sage comb honey bought in the open market failed to protect guinea-pigs from scurvy. Hawk, Smith and Bergheim [1921] were also unable to detect vitamin A, B or C in strained honey, as were Scheunert, Schieblich and Schwanebeck [1923] using a centrifuged lime honey from East Prussia, a comb honey with wax from Lüneberger Heide and a honey not of German origin¹. Great progress has been made in the domain of vitamin research since the above tests were carried out. This communication records the tests for vitamins A, B₁, B₂, C and D on two representative samples of honey as carried out by the more refined methods now available.

EXPERIMENTAL.

One of the samples was a granular honey containing 82.23 % of dry matter and 0.17 % of ash of West Indian origin kindly supplied by Sir Algernon Aspinall. The other sample was an English honey prepared at the Bee Research Institute, Rothamsted, under the supervision of Mr D. Morland, M.A., to whom I should like to express here my indebtedness for the help he extended. The following is Mr Morland's report of the conditions under which the honey was produced.

"The honey was gathered by a stock of Italian bees at Kimpton Hoo, near Welwyn, Herts. A clean super of new frames with wax foundation was given to the colony on July 12th, 1928. At that time the colony had brood in two chambers (British Standard) and had partly filled one super with honey. No queen excluder was used. The weather was favourable throughout the period. The sunshine figures at Rothamsted, which is a few miles from Welwyn, for the period July 12th to July 28th inclusive show a total of 173.8 hours of 'bright sunshine.' White clover was yielding at the time. Much of the neighbourhood is park grassland and the subsoil is chalk. The super was removed

¹ According to the Annual Bureau of Home Economics (1927) two examples of honey examined were found deficient in vitamins A, B, C and D (quoted by Phillips in *Gleanings from Bee Culture*, Jan. 1929).

on July 28th. The hive had increased in weight during this period from 153½ lb. to 232½ lb. The combs used in the feeding experiment were fully sealed over and the honey appeared to be of excellent quality."

This honey when extracted contained 84.95 % of dry matter and 0.10 % of ash.

In order to extract the English honey from the comb the super was mounted horizontally and the sealings of the cells were removed with a hot knife. The wax and honey of these exposed cells were then removed by means of a spoon and filtered through muslin which retained the wax completely. Just sufficient honey for the day's dosing was extracted daily. The West Indian honey was fed without previous treatment.

VITAMIN A.

Vitamin A was estimated by a method based on the Steenbock principle. Young rats weighing 35–40 g. were placed in separate cages on a diet free from the fat-soluble vitamins [Zilva and Miura, 1921]. At the end of 28 days the ordinary cotton-seed oil of the basal diet was replaced by irradiated cotton-seed oil. When the cessation of growth due to the deficiency of vitamin A became apparent, 2 g. of honey were administered daily previous to the delivery of the basal diet. With each test a series of negative controls (on the basal diet alone) and of positive controls receiving 3 drops of cod-liver oil daily were employed. It will be seen from Fig. 1 that the growth curves of the rats which received the honey are similar to those of the negative control animals and consequently a daily dose of 2 g. of honey contained no appreciable quantity of vitamin A.

VITAMINS B₁ AND B₂.

Vitamins B₁ and B₂ were estimated on the principle described by Chick and Roscoe [1927, 1928], whose help and criticism I wish gratefully to acknowledge. The *L* diet described by these authors was employed as the basal diet. The caseinogen used was B.D.H. "fat-free, vitamin-free casein" and not the specially extracted caseinogen used by them. Six groups of rats were used in the tests and were arranged as follows: group 1, basal diet; group 2, basal diet + 2 g. daily of English honey; group 3, basal diet + 2 g. daily of West Indian honey; group 4, basal diet with 0.4 g. autoclaved yeast + 2 g. English honey daily; group 5, basal diet with 0.4 g. autoclaved yeast + 2 g. West Indian honey daily; and group 6, basal diet and 0.4 g. dried yeast daily. The autoclaved yeast was given to supply vitamin B₂, so that in the event of vitamin B₁ being present in the honey the animals receiving this yeast would have shown growth. The animals in all groups received 3–5 drops of cod-liver oil daily as a source of vitamins A and D. Fig. 2 gives the weight curves of the animals in these groups. With the exception of the positive controls in group 6, the animals of which received both vitamins B₁ and B₂ in the dried yeast

Fig. 1. Test for vitamin A in honey.

I
↓ denotes when irradiated diet first received.

K at end of a graph denotes that animal survived the period of 28 days after giving dose.

↓ denotes when animal began to receive honey.

I Positive controls for West Indian honey.

II West Indian honey.

III Negative controls for West Indian honey.

IV Positive controls for English honey.

V English honey.

VI Negative controls for English honey.

Fig. 2. Test for vitamins B₁ and B₂ in honey.

YE

denotes when Peters's extract was first given.

H

denotes stoppage of daily dose of honey.

P

denotes definite symptoms of pellagra.

there was little growth. Rats 61, 64, 65, 67, 69, 71, 75 and 76 were given by Miss Chick and Miss Roscoe after the 34th day a daily dose of 0.1 cc. of Peters's antineuritic extract (0.1 cc. daily equal to 0.7 g. yeast) to supply vitamin B₁, and, as will be seen from the weight curves of these animals, no resumption in growth followed this treatment. It may, therefore, be safely assumed that both samples of honey were deficient in vitamins B₁ and B₂.

VITAMIN C.

Guinea-pigs subsisting on a scorbutic diet of oats, bran, barley meal, middlings and fish meal, which was offered *ad lib.*, and 40–60 cc. of autoclaved milk, were used in this test. The honey was diluted with about one-third of its weight of water and was administered daily to the animal from the commencement of the experiment. The initial daily dose was 3 g. and it was gradually increased. Certain difficulties were encountered with these tests as the guinea-pigs showed a tendency to succumb to an intestinal disturbance in the early stages of the tests and conclusions had to be drawn from such animals as survived long enough for the development of scurvy. The results are summarised in Table I. It is evident from the figures that although death was somewhat accelerated in certain cases by intestinal disturbance, there was not sufficient antiscorbutic potency in the doses given even to delay the onset of the disease.

Table I. *Test for vitamin C in honey.*

Variety of honey	Ref. no. of guinea-pig	Initial wt. g.	Maximum wt. g.	Final wt. g.	No. of days alive	Average daily consumption of honey g.	Day of 1st appearance of scurvy	P.-M. remarks
West Indian	399	315	370	280	27	4.4	15	Died of scurvy
	405	260	295	255	19	5.3	17	Degree of scurvy was such as to indicate that dose was completely negative, though death was due in part to an intestinal disturbance
	406	255	300	220	32	5.1	17	Died of scurvy
	407	260	320	210	28	5.2	17	Died of scurvy
	408	285	287	205	27	5.1	14	Died of scurvy
	409	260	280	250	22	5.4	16	Same condition as no. 405
	410	265	300	240	23	5.3	15	Died of scurvy
	412	285	320	255	22	5.2	14	Scurvy and intestinal disturbance
	413	260	305	200	33	5.1	14	Died of scurvy
English comb	414	260	315	225	29	5.2	14	Died of scurvy
	416	260	260	205	23	4.7	15	Scurvy and intestinal disturbance
	417	260	260	190	25	4.7	18	Died of scurvy
	418	285	290	235	24	5.7	17	Died of scurvy
	419	285	330	255	23	5.9	15	Died of scurvy

VITAMIN D.

This vitamin was determined by the Chick, Korenchevsky and Roscoe [1926] method which is based on the comparison of the ratios of the ash to the organic material, other than those substances extractable by hot alcohol and ether, (*A/R* ratios) of the bones of young rats which have subsisted for about 4 weeks on McCollum's 3143 rickets-producing diet low in phosphorus.

Table II. *Test for vitamin D in honey.**Positive controls for West Indian honey.*

Rat no.	Initial wt. in g.	Final wt. in g.	Weight of bone g.	% water	% fat	% organic residue	% ash	A/R	Average A/R
1 ♀	40	59	0.8407	54.15	4.85	21.54	19.45	0.903	
2 ♂	40	55	0.8139	55.68	6.12	21.26	16.94	0.797	
8 ♂	41	67	1.0236	51.56	6.96	22.70	18.77	0.827	
11 ♂	40	62	0.9800	55.82	6.76	20.79	16.64	0.801	
12 ♂	41	52	0.8590	55.56	5.93	20.96	17.54	0.837	
17 ♀	38	56	0.8048	53.88	4.87	22.23	19.02	0.856	
18 ♂	40	57	0.9040	54.78	6.08	21.79	17.34	0.796	
22 ♂	43	70	0.9396	54.42	6.66	21.09	17.82	0.845	0.833

2 g. West Indian honey daily.

3 ♀	40	66	0.8829	62.04	4.06	22.64	11.28	0.499	
4 ♂	41	75	1.0293	60.33	5.53	22.04	12.12	0.550	
7 ♂	41	72	1.0670	61.33	4.02	23.41	11.23	0.480	
9 ♀	37	56	0.8331	59.99	5.58	21.80	12.63	0.579	
10 ♂	39	67	0.9225	62.93	4.61	21.55	10.91	0.507	
19 ♀	43	70	0.8752	59.44	3.27	22.40	14.89	0.665	
20 ♂	42	74	1.0725	61.08	4.43	21.44	13.04	0.608	
21 ♀	43	75	0.9456	62.34	3.24	23.46	10.96	0.467	0.544

Negative controls for West Indian honey.

5 ♀	41	72	0.9447	63.67	3.12	22.89	10.31	0.451	
6 ♀	41	76	0.9438	63.44	3.78	22.68	10.08	0.445	
13 ♂	41	65	0.9187	63.53	2.78	22.52	11.18	0.497	
14 ♂	40	63	0.9475	62.70	4.98	22.07	10.27	0.465	
15 ♀	43	77	0.9305	61.64	3.63	22.26	12.47	0.560	
16 ♂	42	68	0.9556	63.83	3.33	22.29	10.54	0.473	
23 ♂	44	76	0.9610	59.60	5.00	21.61	13.79	0.638	0.504

Positive controls for English comb honey.

24 ♀	45	59	0.7873	55.49	7.06	21.42	16.02	0.747	
25 ♂	45	57	0.8233	50.79	9.97	20.91	18.31	0.876	
26 ♀	45	57	0.8309	52.36	8.55	21.37	17.72	0.827	
36 ♂	42	69	0.7506	53.26	6.32	21.54	18.87	0.878	
37 ♀	39	60	0.8693	54.80	7.82	21.20	16.18	0.763	
38 ♂	40	67	0.9292	53.30	8.50	21.13	17.07	0.807	0.812

2 g. English comb honey daily.

27 ♂	43	71	0.9546	63.78	5.97	20.84	9.42	0.452	
28 ♀	43	68	0.8510	63.62	3.73	22.84	9.81	0.429	
29 ♂	50	78	0.9449	62.14	4.89	22.84	10.06	0.440	
39 ♀	35	52	0.6489	63.33	4.41	23.93	8.32	0.347	
40 ♀	39	58	0.7509	63.98	4.07	23.03	8.92	0.386	
41 ♀	41	58	0.7524	64.49	3.07	24.64	7.80	0.317	0.395

Negative controls for English comb honey.

30 ♀	43	70	0.8759	62.66	4.69	22.32	10.35	0.464	
31 ♂	45	79	0.9889	62.62	4.49	22.62	10.26	0.454	
32 ♂	48	75	0.9362	61.94	4.43	22.05	11.57	0.525	
33 ♀	39	54	0.7329	62.91	4.06	24.51	8.52	0.348	
34 ♂	41	63	0.8126	65.88	3.62	22.92	7.60	0.333	
35 ♀	38	59	0.7987	64.91	3.42	23.58	8.09	0.343	0.411

The animals received a daily dose of 2 g. of each of the honeys prepared as above. With each honey a group of negative control animals on the basal diet only and a positive group which received 3 drops of cod-liver oil in addition were employed. As will be seen from Table II the differences between the A/R ratio of the rats receiving the honeys and those of the corresponding negative control animals are of an order which falls within the limits of accuracy of the method. It may be safely assumed that both the honeys were inactive in the doses employed in the test.

CONCLUSION.

Tests carried out on a fresh English sample of comb honey and on a West Indian honey show that both samples are deficient in vitamins A, B₁, B₂, C and D. These results are in consonance with those obtained by other workers, which show that honey is not a source for these vitamins and that this deficiency is not due to deterioration consequent on treatment or storage.

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