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HONEY AS A CARRIER OF INTESTINAL DISEASES

By
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HONEY AS A CARRIER OF INTESTINAL DISEASES

By
WALTER G. SACKETT

Among the different articles of food which we have come to regard as possible carriers of the germs of the typhoid-colon group, no one appears to have suggested honey. This is rather surprising, considering the fact that it is consumed in a raw state, and that its occurence and use are quite general. The failure to include honey in the list is probably because the majority of investigators who are devoting their time to studies of this kind are not familiar either with the conditions under which honey is procured or with the habits of honey bees.

While pursuing another investigation in Tennessee during July, 1918, the writer frequently observed bees crawling over the human excrement of the family privy. This structure was quite typical of the farm privies of that section; no excavation had been made to receive the fecal matter, and to facilitate the removal of the latter, the lower part of the back side was not boarded up. Chickens, snakes, flies, and bees, all had free access thereto, and among them but little material was allowed to accumulate. Subsequent inquiry concerning this peculiar habit of honey bees has confirmed the writer's observations.

It is difficult to conceive of conditions better suited to the dissemination of intestinal bacteria than those afforded by the case just cited, and if an ideal opportunity was ever offered for the contamination of a food stuff, it should have been realized in the wax and honey produced by such bees. The possible relation of this subject to food poisoning seemed of sufficient importance to justify an inquiry into the behavior of intestinal bacteria in honey, and accordingly we have undertaken a study of the longevity of ten micro-organisms of this group in extracted honey.

MICRO-ORGANISMS OF THE APIARY

Before considering the experimental work with which this paper deals, a brief reference to the micro-organisms which have been found associated with bees and their products will be of interest.

FROM COMBS

White[1] has isolated *Bacillus A* (*Bacillus mesentericus?*), *Bact. acidiformans*, and *Saccharomyces roseus* from combs.

FROM POLLEN AND INTESTINE OF HEALTHY HONEY BEES

From the pollen and intestine of the bee, White[1] has separated a motile rod, designated *Bacillus B* described briefly as follows:

Motile, Gram negative rod which liquefies gelatin slowly; egg yellow pigment; alkaline reaction in litmus milk; acid but no gas from dextrose; slight acid production from maltose and mannite; alkaline reaction with lactose, saccharose and levulose.

FROM COMB HONEY AND HEALTHY LARVAE

The same author states that he has examined comb honey from a large number of sources and has found it to be quite uniformly sterile. Healthy larvae are likewise sterile. The writer has made similar examinations of comb honey from the local market and his findings confirm those of White.

UPON ADULT BEES

White has noted *Bacillus A* (*B. mesentericus?*), *Bact. cyaneus* (*Micrococcus cyaneus*) and *Micrococcus C* as occurring upon adult bees.

Micrococcus C.—Non motile, Gram positive coccus; liquefies gelatin; produces acid from dextrose, lactose, saccharose, levulose, maltose, and mannite, but no gas; milk is coagulated and the casein is liquefied.

IN INTESTINE OF HEALTHY HONEY BEE

According to White, the temperature of the healthy honey bee approximates that of warm blooded animals, and many of the same bacteria that occur in the bee's intestine are also found in the intestine of man and other animals. The number of species in any individual is comparatively small, altho the number of organisms may be large.

The list includes:

Bacillus cloacae	*Bacillus cholerae suis*
Bacillus coli communis	*Bacillus sugastricus*
Bact. mycoides	*Ps. fluorescens liquefaciens*
Bacterium D.	*Bacillus E.*
Saccharomyces F.	*Saccharomyces G.*
Saccharomyces roseus	

Bacterium D.—Non-motile rod, non-spore-forming anaerobe; does not liquefy galatin; produces neither acid nor gas from dextrose.

Bacillus E.—Motile rod; does not produce spores, Gram positive; liquefies gelatin slowly; lemon yellow in color; produces acid from dextrose, saccharose, mannite; reaction alkaline with lactose, levulose, and maltose. Milk coagulated and casein slightly digested.

Bacillus alvei, believed for a long time to be the cause of European foul brood, is frequently found in dead larvae. Pollen stored in foul brood combs and likewise honey in brood combs infected with the disease contain relatively few germs. The surface of the combs, frames, and hives may be contaminated with it as well as the wings, head, legs, thorax, abdomen, and intestinal contents of adult bees.

Bacillus pluton, the true cause of European foul brood, according to White[2], and *Bacillus larvae,* the organism responsible for American foul brood, appear to be present in the honey of infected hives. In evidence of this, we find such statements as the following:—

"The honey from a diseased colony should be diluted to prevent burning and then thoroly sterilized by hard boiling for at least one-half hour, if it is to be fed back to the bees".[3]

"In order to kill the bacteria of either of the brood diseases, it is desirable to dilute the honey by adding an equal amount of water and then raising the temperature to the boiling point and keep it there, allowing the mixture to boil vigorously for at least 30 minutes; in order that no risk may be run, it is better to make this one hour".[3]

"The common means of carrying the virus is in honey which has become contaminated".[4]

Fermented and sour honey furnish additional evidence that micro-organisms are capable of living and developing in this medium. The exact nature of the fermentation appears to be somewhat uncertain, but it is probably both alcoholic and acid (acetic). These changes are observed most frequently in unripe honey, that in which the moisture content is more than 25 per cent. Such honey usually contains more succrose than the finished product and during the ripening process this is converted into invert sugar.

Shutt[5] states that honey from uncapped or only partially capped combs is usually immature, containing a higher percentage of moisture and having decidedly *poorer keeping qualities* than honey from fully capped combs.

Root[6], in commenting on *fermented* honey, says that "Probably not one beekeeper in a hundred can tell by the taste or appearance whether extracted honey when put up in cans and kept in a dry place is proof against *fermentation.** Part of a lot of honey, (granulated hard when first bought) tho kept in a warm, dry room, *fermented* and *expanded* the cans until they burst. This honey was not extremely sour to the taste, and yet there was a very perceptible flavor of *fermentation*, practically ruining the whole lot".

Again we read[7]:

"Can you give me a remedy for honey *souring* in the hive? . . . . It begins with a few small air bubbles in the cells, which increase in size and number

*Italics by the writer.

until the cells are full, and a perceptible movement is obvious. This honey is very thin and sour".

Cook[7], in commenting on this phenomenon, attributes the trouble to bacteria, adding that,

"In the action of these germs, not only is acetic acid produced, but, as surely, carbon dioxide gas. Barrels of unripe honey are apt to burst from this cause".

Nussbaumer[8] has found several species of *zygosaccharomycetes* in honey, capable of inducing fermentation.

Farnsteiner[9] has studied the changes produced in sour honey by pasteurization.

Browne[10], who has made a very thoro study of the chemical composition of American honeys, explains the keeping quality of ripe honey, in a measure at least, thus:

"Another modification produced in the nectar by the bees is the introduction of a minute quantity of formic acid. This acid is wanting in the pollen and nectar of flowers and is supposed to be introduced into the honey by the bee just previous to capping the cell. The formic acid thus introduced by the bee is supposed to act as a *preservative and prevent the honey from fermenting*".

These citations to the fermentation of honey have been introduced here as prima facie evidence in support of the contention that certain micro-organisms, at least, are able *to live in honey* and when once established *can bring about well recognized chemical changes.*

Browne gives the following composition of American honey:

Invert sugar	74.44 per cent.
Succrose	1.90 per cent.
Moisture	17.59 per cent.

Acidity (expressed as formic acid) varies from .04 to .25 per cent. with an average of .09 per cent.

To find bacteria thriving in as concentrated a solution as is represented by this analysis is rather unexpected, yet the facts in the case seem to bear this out with respect to the organisms mentioned above.

The fate of the members of the typhoid-colon group in this medium will be considered in the pages which follow.

PRESENT WORK

HONEY.—Extra fine, extracted, alfalfa honey, obtained directly from the producer in stock, five-pound tin pails, was used in the study. It was very light in color and had been heated but slightly to facilitate the extraction. It crystallized readily at 20° C. and was weighed in this form for the different dilutions employed.

The acidity, expressed as formic acid, was .11 per cent. for Lot No. 1 and .09 per cent. for Lot No. 2.

METHODS

DILUTIONS OF HONEY.—In order to determine the effect of the concentration of the honey upon the bacteria studied, the proper weights of crystallized honey were added to physiological salt solution (.85 per cent. NaCl in distilled water) to give the following percentage dilutions: 0.0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100.

25 c.c. portions of each dilution were measured into 100 c.c. Erlenmeyer flasks and sterilized for 30 minutes in flowing steam on three consecutive days.

CULTURES.—The following organisms were used in the experiment:

B. typhosus
B. fecalis alkaligenes
B. proteus vulgaris
B. suipestifer
B. lactis aerogenes
B. paratyphosus "B"
B. paratyphosus "A"
B. coli communis
B. dysenteriae
B. enteritidis

Suspensions of these were made in physiological salt solution from 48-hour agar strokes, and 1 c.c. of each was added to each flask of honey as an inoculum. The flasks were kept at room temperature.

To ascertain the approximate length of time that the cultures were able to survive in the honey, agar plates were made from the flask cultures, using one loop for the transfer, when the honey was first inoculated, after 5 and 10 hours, and daily thereafter for 12 days. In some cases the plating was continued for a longer period.

SERIES I.—NEW FALL HONEY

TABLE 1.—BACILLUS TYPHOSUS

Per cent. Honey	Longevity of Culture													
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.	40d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	±*	—						
20.0	+	+	+	+	+	+	—							
30.0	+	+	+	+	+	+	—							
40.0	+	+	+	+	+	+	—							
50.0	+	+	+	+	+	—								
60.0	+	+	+	+	—									
70.0	+	+	+	+	—									
80.0	+	+	+	+	—									
90.0	+	+	+	+	—									
100.0	+	+	+	+	+	—								

*NOTE.—The character ±, wherever it occurs in the above and following tables, denotes a very marked reduction in the number of bacteria.

In the pure honey, *B. typhosus* remained alive for 48 hours. After 24 hours it was dead in dilutions above 50 per cent.; after 48 hours it had disappeared from the 50 per cent.; after 3 days it was alive only in the 10 per cent., and in the salt solution control, and after 4 days it was present only in the control, where it was still alive after 40 days.

TABLE 2.—BACILLUS FECALIS ALKALIGENES

Per cent. Honey	Longevity of Culture					
	0	5 hrs.	10 hrs.	1d.	2d.	40d.
0.0	+	+	+	+	+	+
10.0	+	+	+	—		
20.0	+	+	—			
30.0	+	+	—			
40.0	+	+	—			
50.0	+	—				
60.0	+	—				
70.0	+	—				
80.0	+	—				
90.0	+	—				
100.0	+	—				

B. fecalis alkaligenes appears to be the most sensitive of all the organisms studied. It was killed by all concentrations above 50 per cent., including pure honey, inside of 5 hours and after 10 hours remained alive only in the 10 per cent. and control; at the end of 24 hours it was present only in the control, where it was still alive after 40 days.

TABLE 3.—BACILLUS PROTEUS VULGARIS

Per cent. Honey	Longevity of Culture															
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.	15d.	20d.	25d.	40d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*
20.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*
30.0	+	+	+	+	+	+	+	±	—								
40.0	+	+	+	+	+	—											
50.0	+	+	+	+	—												
60.0	+	+	+	+	—												
70.0	+	+	+	+	—												
80.0	+	+	+	+	—												
90.0	+	+	+	+	—												
100.0	+	+	+	+	+	+	±	—									

Bacillus proteus vulgaris was present in all concentrations during the first 24 hours; it died out in the pure honey after the fourth day. On the second day it had disappeared from all dilutions above 40 per cent., and on the third day it was absent from

* Discontinued.

the 40 per cent. There was a very marked reduction in the number of bacteria in the 30 per cent. on the fifth day and all were dead on the sixth. The culture remained alive in the 10 per cent. and 20 per cent. for 25 days, at which time the platings were discontinued. The control culture in salt solution was still alive after 40 days.

TABLE 4.—BACILLUS SUIPESTIFER

Per cent. Honey	Longevity of Culture														
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.	11d.	40d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	+	+	+	+	+	+	±	—	
20.0	+	+	+	+	+	+	+	+	+	—					
30.0	+	+	+	+	+	+	+	+	—						
40.0	+	+	+	+	+	+	—								
50.0	+	+	+	+	+	—									
60.0	+	+	+	+	±	—									
70.0	+	+	+	+	±	—									
80.0	+	+	+	+	±	—									
90.0	+	+	+	+	±	—									
100.0	+	+	+	+	±	±	—								

Bacillus suipestifer survived all concentrations for 24 hours, but the number of bacteria was greatly reduced in the 60, 70, 80, and 90 per cent. in 48 hours; the culture was dead in the pure honey on the fourth day. On the third day all dilutions were dead above 40 per cent.; by the eighth day, the 10 per cent. and the control alone gave growth, the former showing a great reduction in numbers on the 10th day and complete disappearance on the eleventh. The control was still alive after 40 days.

TABLE 5.—BACILLUS LACTIS AEROGENES

Per cent. Honey	Longevity of Culture																
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.	11d.	20d.	25d.	40d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
20.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
30.0	+	+	+	+	+	+	+	+	+	+	+	+	+	—			
40.0	+	+	+	+	+	+	±	—									
50.0	+	+	+	+	±	±	—										
60.0	+	+	+	+	±	—											
70.0	+	+	+	+	±	—											
80.0	+	+	+	+	±	—											
90.0	+	+	+	+	±	—											
100.0	+	+	+	+	+	±	—										

Bacillus lactis aerogenes was present in all concentrations after 24 hours, but showed a very marked reduction in numbers in 48 hours above 40 per cent.; it died out in the pure honey on the

fourth day. On the third day it disappeared from the 60, 70, 80, and 90 per cent., and on the fourth, fifth, and eleventh days from the 50 per cent., 40 per cent., and 30 per cent., respectively. Abundant growth was obtained from the control, 10 per cent., and 20 per cent. after 25 days, but in 40 days all were dead but the control.

TABLE 6.—BACILLUS PARATYPHOSUS "B"

Per cent. Honey	Longevity of Culture								
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	40d.
0.0	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	±	—		
20.0	+	+	+	+	+	—			
30.0	+	+	+	+	—				
40.0	+	+	+	—					
50.0	+	+	+	—					
60.0	+	+	±	—					
70.0	+	+	±	—					
80.0	+	+	±	—					
90.0	+	+	±	—					
100.0	+	+	+	+	—				

Bacillus paratyphosus "B" was among the most sensitive organisms studied. After 10 hours its numbers were very much reduced in the 60, 70, 80, and 90 per cent. In 24 hours it had disappeared altogether from dilutions above 30 per cent.; no growth was obtained from the 30 per cent. on the second day, and on the third the organisms had died out in the 20 per cent. and were greatly diminished in the 10 per cent., from which it was absent on the fourth day. The control in salt solution was alive on the fortieth day. It disappeared from the pure honey in 48 hours.

TABLE 7.—BACILLUS COLI COMMUNIS

Per cent. Honey	Longevity of Culture															
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.	11d.	12d.	40d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	+	+	+	+	+	+	±	±	—	
20.0	+	+	+	+	+	+	+	±	±	—						
30.0	+	+	+	+	+	+	+	—								
40.0	+	+	+	+	+	+	±	—								
50.0	+	+	+	+	+	+	—									
60.0	+	+	+	+	+	+	—									
70.0	+	+	+	+	+	+	—									
80.0	+	+	+	+	+	+	—									
90.0	+	+	+	+	+	+	—									
100.0	+	+	+	+	+	+	±	±	—							

Bacillus coli communis was the most resistant form met with. No harmful action could be noted in any concentration until the fourth day, when the culture was dead in the 50, 60, 70, 80, and

90 per cent. solution. At this time there was also considerable reduction in the 40 per cent. On the fifth day no growth was secured from the 40 and 30 per cent., and the numbers were much less in the 20 per cent.; the latter was sterile on the seventh; the culture was still alive in the 10 per cent. on the eleventh day, but was dead on the twelfth. The control gave good growth still on the fortieth day. It was dead in the pure honey on the sixth day.

TABLE 8.—BACILLUS DYSENTERIAE

Per cent. Honey	Longevity of Culture								
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	25d.	30d.
0.0	+	+	+	+	+	+	+	+	—
10.0	+	+	+	+	+	+	—		
20.0	+	+	+	+	+	±	—		
30.0	+	+	+	+	—				
40.0	+	+	—						
50.0	+	+	—						
60.0	+	+	—						
70.0	+	+	—						
80.0	+	+	—						
90.0	+	+	—						
100.0	+	+	—						

Next to *Bacillus facilis alkaligenes*, *Bacillus dysenteriae* appears to be the most sensitive to the injurious action of the honey. Ten hours exposure to the 40, 50, 60, 70, 80, and 90 per cent. dilutions, as well as the pure honey, was sufficient to destroy this organism. It died out on the second day in the 30 per cent. and on the fourth day in the 10 and 20 per cent. The control still gave growth after 25 days, but was dead after 30 days.

TABLE 9.—BACILLUS ENTERITIDIS

Per cent. Honey	Longevity of Culture															
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.	15d.	25d.	40d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
20.0	+	+	+	+	+	+	±	±	—							
30.0	+	+	+	+	+	+	±	—								
40.0	+	+	+	+	±	—										
50.0	+	+	+	±	—											
60.0	+	+	+	±	—											
70.0	+	+	+	±	—											
80.0	+	+	+	±	—											
90.0	+	+	+	±	—											
100.0	+	+	+	±	—											

Bacillus enteritidis showed the injurious action of the honey after 24 hours in the dilutions above 40 per cent., the same giving no growth in 48 hours. It was dead in the pure honey in 48 hours;

numbers were reduced in the 40 per cent. in 48 hours, and the culture was dead on the third day. On the fourth day the organisms in both the 30 per cent. and 20 per cent. were affected, and they died out in the former on the fifth day and in the latter on the sixth. The bacteria were still active in the 10 per cent. dilution after 25 days, but were dead in 40 days. The control was alive after 40 days.

TABLE 10.—BACILLUS PARATYPHOSUS "A"

Per cent. Honey	Longevity of Culture													
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.	40d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	+	+	±	±	—			
20.0	+	+	+	+	+	—								
30.0	+	+	+	+	—									
40.0	+	+	+	—										
50.0	+	+	+	—										
60.0	+	+	+	—										
70.0	+	+	+	—										
80.0	+	+	+	—										
90.0	+	+	+	—										
100.0	+	+	+	—										

Bacillus paratyphosus "A" appears to exhibit about the same degree of injury from the higher concentrations as *B. paratyphosus "B"*. The culture was dead after 24 hours in all dilutions above 30 per cent., including that in the pure honey. It disappeared from the 30 per cent. and 20 per cent. on the second and third days, respectively, and was reduced in numbers in the 10 per cent. by the sixth day, disappearing altogether on the eighth. The control in salt solution was alive after 40 days.

SERIES II.—FALL HONEY SIX MONTHS OLD

A second series of experiments was undertaken with another lot of honey, which differed from the first only in that it had stood six months in an open-top pail and had lost water by evaporation. The acidity of this sample was .09 per cent. computed as formic acid.

TABLE 11.—BACILLUS TYPHOSUS

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	—							
20.0	+	+	+	+	—								
30.0	+	+	+	—									
40.0	+	+	+	—									
50.0	+	+	+	—									
60.0	+	+	—										
70.0	+	+	—										
80.0	+	+	—										
90.0	+	+	—										
100.0	+	+	+	—									

B. typhosus was no longer present in the pure honey after 24 hours, and it was absent from the dilutions above 50 per cent. in 10 hours. After 24 hours it had disappeared from the 30, 40, and 50 per cent. dilutions; after 48 and 72 hours it was no longer present in the 20 and 10 per cent. dilutions, respectively. The control was still alive after 10 days.

TABLE 12.—B. FECALIS ALKALIGENES

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	—										
20.0	+	—											
30.0	+	—											
40.0	+	—											
50.0	+	—											
60.0	+	—											
70.0	+	—											
80.0	+	—											
90.0	+	—											
100.0	+	—											

B. fecalis alkaligenes was even more sensitive to the killing action of the honey here than in the preceding series. After 5 hours it had disappeared from all dilutions except the 10 per cent. and was absent from this after 10 hours. The pure honey was sterile after 5 hours. The control was alive after 10 days.

TABLE 13.—B. PROTEUS VULGARIS

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	+	+	+	+	+	+	+
20.0	+	+	+	+	+	+	+	+	+	+	+	+	+
30.0	+	+	+	+	—								
40.0	+	+	+	—									
50.0	+	+	+	—									
60.0	+	+	+	—									
70.0	+	+	+	—									
80.0	+	+	+	—									
90.0	+	+	+	—									
100.0	+	+	+	+	+	±	—						

B. proteus vulgaris was the most resistant of all the organisms in the lowest concentrations, being present in apparently undiminished numbers in the control, 10, and 20 per cent. after 10 days. It was dead in the pure honey on the fourth day; after 24 hours it had disappeared from all dilutions above the 30 per cent., which was sterile on the second day.

TABLE 14.—B. SUIPESTIFER

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	±	—						
20.0	+	+	+	+	—								
30.0	+	+	+	+	—								
40.0	+	+	+	—									
50.0	+	+	+	—									
60.0	+	+	—										
70.0	+	+	—										
80.0	+	+	—										
90.0	+	+	—										
100.0	+	+	+	+	±	—							

In the pure honey, *B. suipestifer* was still alive on the second day, altho decreased in numbers, but by the third it was dead. In dilutions above 50 per cent. it was dead after 10 hours; it was absent in 24 hours from the 40 and 50 per cent., and in 48 hours it had disappeared from the 20 and 30 per cent. On the third day the numbers were considerably reduced in the 10 per cent., and on the fourth day the solution was sterile. The control was alive after 10 days.

TABLE 15.—B. LACTIS AEROGENES

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	+	+	+	+	+	+	+	+
20.0	+	+	+	+	+	+	±	±	±	±	—		
30.0	+	+	+	+	—								
40.0	+	+	+	—									
50.0	+	+	+	—									
60.0	+	+	±	—									
70.0	+	+	±	—									
80.0	+	+	±	—									
90.0	+	+	±	—									
100.0	+	+	+	+	±	—							

B. lactis aerogenes lived in the pure honey until the third day; however, in 48 hours there was a reduction in numbers. The organisms disappeared rather rapidly from the 60, 70, 80, and 90 per cent. in 10 hours and were absent from all above 30 per cent. in 24 hours. After 48 hours the 30 per cent. gave no growth and by the eighth day the 20 per cent. was sterile; growth was still obtained from the 10 per cent. and control after 10 days.

TABLE 16.—PARATYPHOSUS "B"

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	±	—							
20.0	+	+	+	+	—								
30.0	+	+	—										
40.0	+	+	—										
50.0	+	+	—										
60.0	+	±	—										
70.0	+	±	—										
80.0	+	±	—										
90.0	+	±	—										
100.0	+	+	+	—									

B. paratyphosus "B" behaved almost the same toward the honey as *B. paratyphosus "A"*. The germs were dead in 24 hours in the pure honey; the numbers were much reduced in the 60, 70, 80, and 90 per cent. after 5 hours, and in 10 hours all were dead in the dilutions above 20 per cent. After 48 hours no growth was obtained from the 20 per cent. and there was a very marked reduction in numbers in the 10 per cent.; the latter was sterile on the third day. The control was alive after 10 days.

TABLE 17.—B. COLI COMMUNIS

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	±	±	±	—				
20.0	+	+	+	+	+	±	—						
30.0	+	+	+	+	+	—							
40.0	+	+	+	+	+	—							
50.0	+	+	+	+	±	—							
60.0	+	+	+	+	±	—							
70.0	+	+	+	+	±	—							
80.0	+	+	+	+	±	—							
90.0	+	+	+	+	±	—							
100.0	+	+	+	+	+	±	±	—					

As in the preceding series, *B. coli communis* was the most resistant organism studied so far as the higher concentrations were concerned. It did not die out in the pure honey until the fifth day, altho there was a reduction in numbers on the third day. On the second day there was a reduction in numbers in all concentrations above 40 per cent., while by the third, all were dead above 20 per cent. The 20 per cent. died out by the fourth day, and the 10 per cent. was sterile on the sixth. The control was alive after 10 days.

TABLE 18.—B. DYSENTERIAE

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	±	—										
20.0	+	±	—										
30.0	+	—											
40.0	+	—											
50.0	+	—											
60.0	+	—											
70.0	+	—											
80.0	+	—											
90.0	+	—											
100.0	+	+	—										

B. dysenteriae appeared to be almost as sensitive as *B. fecalis alkaligenes*. It was present in the pure honey after 5 hours, but absent after 10. In 5 hours the organisms had disappeared from all dilutions above the 20 per cent. and the numbers were much reduced in the 10 and 20 per cent.; after 10 hours, these, too, were sterile. The control gave growth after 10 days.

TABLE 19.—B. ENTERITIDIS

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	±	—								
20.0	+	+	+	—									
30.0	+	+	+	—									
40.0	+	+	+	—									
50.0	+	+	+	—									
60.0	+	+	±	—									
70.0	+	+	±	—									
80.0	+	+	±	—									
90.0	+	+	±	—									
100.0	+	+	+	+	±	—							

B. enteritidis was alive in the pure honey on the second day, but its numbers were much less than in the control; it was dead on the third day. There was a marked reduction of the organisms in the 60, 70, 80, and 90 per cent. concentrations in 10 hours, and after 24 hours, the 10 per cent. dilution was the onlv one which contained living organisms; by the second day they had disappeared from this. The control gave growth after 10 days.

TABLE 20.—B. PARATYPHOSUS "A"

Per cent. Honey	Longevity of Culture												
	0	5 hrs.	10 hrs.	1d.	2d.	3d.	4d.	5d.	6d.	7d.	8d.	9d.	10d.
0.0	+	+	+	+	+	+	+	+	+	+	+	+	+
10.0	+	+	+	+	+	—							
20.0	+	+	+	+	—								
30.0	+	+	—										
40.0	+	+	—										
50.0	+	±	—										
60.0	+	±	—										
70.0	+	±	—										
80.0	+	±	—										
90.0	+	±	—										
100.0	+	+	+	—									

B. paratyphosus "A" was dead in the pure honey after 24 hours. In the dilutions above 40 per cent. the numbers were reduced after 5 hours, and in ten hours everything above 20 per cent. was sterile. By the second day the organisms had disappeared from the 20 per cent., and by the third from the 10 per cent. The control was alive after 10 days.

SUMMARY

The longevity of the typhoid-colon group in honey is very limited.

The failure of the organisms to die out as readily in the concentrated honey as in the dilutions was rather surprising. A possible explanation of this suggests itself in the physical state of the sugar particles in the honey. Assuming the honey to have been a saturated solution, and this appears to have been the case, there is a probability that we had here a colloidal solution with low osmotic pressure. In such a solution, plasmolysis would take place relatively slowly. When water was added, as in the dilutions, some of the colloidal sugar passed over into molecular solution, the osmotic pressure increased and plasmolysis became more active.

B. fecalis alkaligenes succumbs most readily, and *B. coli communis* least so. The other members of the group occupy an intermediate position, increasing in resistance in the higher concentrations in approximately the following order, there being some queston about the relative placing of *B. typhosus, B. enteritidis,* and *B. proteus vulgaris*: *B. dysenteriae, B. paratyphosus "B", B. paratyphosus "A", B. typhosus, B. enteritidis, B. proteus vulgaris, B. suipestifer* and *B. lactis aerogenes.*

The probability of honey acting as a carrier of typhoid fever, dysentery, and varous diarrhoeal affections is very slight.

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