Reports

Do the Nurse Honey Bees Recognize the Sex of the Larvae?

The queen in the honey-bee colony lays two types of eggs, fertilized and unfertilized. Usually the fertilized eggs are laid in the smaller (worker) cells or in the queen cells and produce either workers or queens. The unfertilized eggs are deposited in larger cells and produce drones. The food which the nurse bees fed to queen larvae is almost devoid of pollen; a little pollen is present in the food of older worker larvae, but the food of older drone larvae contains considerable amounts of pollen.

Planta (*1*) did not find any pollen in the food of older worker larvae. However, the food of drone larvae, over 4 days old, showed a great admixture of pollen grains (15,000 grains in 1 mg of food). On microscopical examination, Haydak and Vivino (*2*) found 9 to 11 grains of pollen per field of vision in the food of older worker larvae, and Haydak (*3*) counted an average of 38 grains in the food of older drone larvae. It appears that the nurse bees differentiate between the drone and the worker larvae. Is the sex of the larvae or is the size of the cells instrumental in this differentiation? Gontarski (*4*) considers that not the cell content (the type of the larvae) but the form and the size of the cells are the stimuli determining the type of food deposited in the cells by the nurse bees.

It is a known fact (*5*) that, when offered only drone combs, the queen will lay fertilized eggs in the drone cells. In the presence of worker combs, the queen begins to lay normally, depositing fertilized eggs in the worker, and unfertilized in the drone, cells. On the basis of this knowledge, the following experiment (*6*) was designed.

In the spring (9 May) two queenright packages were hived on drone combs and drone foundation. The queen started egg laying, and the larvae were fed normally. The food was then taken from the cells that contained the older larvae. The lower walls of the cells were destroyed, the larvae were removed, and the food was taken out with the help of a royal-jelly spoon. Usually five vials were used, the food of ten cells being placed in each. The content of each vial was thoroughly mixed. Five samples were taken from each vial, and five readings were made on each sample, under an objective lens of $\times 44$ magnification. The average pollen count per field of vision was 12 grains.

The larvae were sealed by the bees, with worker-shape cappings, and worker pupae were found in the cells. Ten days later a second set of samples was taken, from the drone cells that contained worker larvae, and the average pollen count was three grains of pollen per field of vision. At this time, about half of the drone combs were taken out, and worker combs were added to the colonies. When older worker larvae were found in the worker cells, the samples of food of older larvae were taken from the worker cells and from the drone cells containing worker larvae. The average pollen count was four grains of pollen for the food of older worker larvae from the drone cells and five grains of pollen for that from the worker cells containing older worker larvae. When the colony started to rear drone larvae in the drone combs, the samples of food from the cells that contained older drone larvae averaged 16 grains per field of vision.

The queens were removed from the colonies. When laying workers appeared, samples of the food from the drone cells that contained older drone larvae were taken again. The average count was six grains of pollen per field of vision. The food from the queen cells built over the drone larvae (which changed to drone pupae in the constant temperature chamber) contained 0.3 grain of pollen per field of vision.

The results seem to indicate that, at the beginning, the bees hesitated somewhat in recognizing the sex of the larvae in the drone cells, thus supplying the older worker larvae with a larger amount of pollen. However, later, they were giving the food for worker larvae, containing less pollen, to the older worker larvae reared in both the drone and the worker cells. When the unfertilized eggs were laid in the drone cells, the bees recognized the drone larvae and fed the older larvae a ration that contained increased amounts of pollen, as is done normally. This would indicate that the nurse bees, under normal conditions, recognized the sex of the larvae irrespective of the size of the cells.

An entirely different picture was observed when the colonies became hopelessly queenless and the laying workers began their activity. In this case the older drone larvae in the drone cells were fed the food containing less pollen, which is normally offered to the older worker larvae. Moreover, the drone larvae in the queen cells received the royal jelly which is given to normal queen larvae. Thus it appears that, in a laying workers' colony, the bees did not differentiate with respect to the sex of the larvae. The cause of this phenomenon is difficult to explain at present.

From these findings it appears that, in the queenright colonies, the nurse bees recognize the sex of the larvae and feed the older larvae of both sexes accordingly. However, in the hopelessly queenless colonies, it seems that the nurse bees feed the older drone larvae as if they were female larvae.

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Upstream Bottom Currents in New York Harbor

Analysis of data obtained during the 1952 current surveys in New York Harbor by the Coast and Geodetic Survey reveal the net upstream movement of large volumes of water near the bottom. These results were possible because the accurate determination of the flood and ebb currents, made it possible to calculate the flow of the nontidal or residual currents flowing in the same direction. This report explains the method whereby

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the volume of flow for nontidal currents was calculated, discusses the upstream flow near the bottom, and shows how variations in fresh-water inflow may be inferred from current measurements alone.

Current measurements were made with the Roberts radio current meter (*1*). Observations of current velocity and direction were recorded every half hour, usually for at least 100 hours, at each of 39 stations, with meters suspended generally at one-fourth, one-half, and three-fourths the charted water depth. This report concerns only the stations in four profiles across New York Harbor and the Hudson River. The Sandy Hook profile consisted of seven stations across the mouth of the harbor from Sandy Hook to Rockaway Point. The Governors Island profile consisted of seven stations across the harbor south of the tip of Manhattan and 12 miles from the harbor mouth. The Riverdale and West Point profiles, of three stations each, were located in the Hudson River, 27 and 56 miles, respectively, upstream from the harbor mouth.

Reversing tidal currents flowed upstream (flood) and downstream (ebb) past each meter, reversing direction four times each tidal day (24 hr, 50 min). The net movement of water throughout a tidal cycle was determined at each meter location as follows: current curves were constructed in which the half-hourly velocities over approximately 100 hours were plotted as ordinates and times were plotted as abscissas. These curves were reduced to obtain mean values for flood and ebb velocities at strength, and for flood and ebb durations. Since the current velocity curve approximates a cosine curve, the mean value of all ordinates within any flood or ebb cycle is equal to $2/\pi$ (or 0.637) times the maximum ordinate of the curve. Thus the mean velocity of the current throughout the flood and ebb periods was taken as 0.637 times the velocity at strength. Multiplying the mean flood or ebb velocity by the duration of flood or ebb gave mean values, over the observation period, for the flood excursion and the ebb excursion, and the difference between these two values indicated the net movement per tidal cycle at that meter. This value was determined for each meter in each of the four cross sections. Each cross section was drawn to scale, and the area of the plane of the cross section represented by each meter was determined by drawing grid lines midway between adjacent meters. This area was multiplied by the net movement per tidal cycle to give the net upstream or downstream volume moved through each segment of each cross section during one tidal cycle. These calculations are based on the assumption that all the water flowing through each segment flows at the rate measured by the meter within that segment. The results are given in Table 1.

Table 1. Volume of flow measured in each of four cross sections. Upstream and downstream values indicate the sums of all segments showing net movement in those directions.

Volume of flow (1000 ft³/sec)				
Upstream	Downstream	Net downstream	Date of survey (1952)	Mean date
		Sandy Hook		
17.0	78.0	61.0	5/28–6/7	6/2
		Governors Island		
19.1	70.1	51.0	6/10–6/23	6/16
		Riverdale		
5.9	40.8	34.9	5/24–5/28	5/26
		West Point		
2.1	20.0	17.9	5/20	
0.0	34.7	34.7	5/21	
0.0	38.9	38.9	5/22	
0.0	51.5	51.5	5/23	

The Sandy Hook cross section showed that, during the observations, nontidal currents flowed in opposite directions in different portions of the cross section. The downstream movement was concentrated in the upper central portions of the stream, whereas the upstream current was noted at the bottom meter at all but the shallowest of the seven stations in this section. The two end stations in the section showed net up-harbor movement also at the surface, reflecting a localization of the surface flood currents. Nontidal upstream flow amounted to 17,000 ft³/sec. At the Governors Island cross section, the nontidal upstream bottom current was found only at the two deepest meters, and the net upstream bottom flow was 19,100 ft³/sec. An upstream bottom current was also found 27 miles upriver in the Riverdale cross section. Here, however, the bottom flow was only 5900 ft³/sec, again at the two deepest meters. At West Point, 29 miles farther up the Hudson, a net upstream bottom current of only 2100 ft³/sec was noted the first day of the series, and net movement was downstream at all depths on the following three days.

The water brought into the harbor along the bottom obviously is not accumulating there, so it must be mixing with the overriding, outflowing Hudson River water and returning to the sea. The mechanism whereby sea water is "pumped" in along the bottom of an estuary has been described experimentally (*2*) and has been observed in other estuaries (*3*). In the present case, however, no salinity measurements were made, and the calculations were possible only because of the completeness of the current surveys.

Since the inflowing bottom water must be returned, then comparable volumes must also be flowing out, mixed with the overriding river water, and the difference between the upstream and downstream flow must therefore be a measure of the river flow. Table 1 shows that this difference was not uniform during the surveys and suggests that there were large changes in the volume of river flow. At three of the four profiles, stations were not all observed simultaneously but rather in two separate groups, so that values for the net downstream movement must be referred to the mean time of the observations. At West Point, however, all stations were observed each day for four days, so that daily values for the net downstream movement could be computed. These were compared with the total daily volume of river flow at the eight Geological Survey gaging stations which measure the flow that eventually passes through the West Point section (*4*). The river flow at West Point, computed from the current observations, showed a steady increase during the four days 20 to 23 May to a high of 51,500 ft³/sec (Table 1). This most closely resembles an increase at the gaging stations during the four days 10 to 13 May to a high of 56,700 ft³/sec (*4*), indicating a lag of 10 days between gaging and the flow past West Point. Seventy-three percent of the water flowing through this profile was measured at the Green Island gaging station 95 miles farther upriver, indicating a rate of advance of 9.5 miles per day for the largest part of the flow. A comparison of the gaging station records with the computed net downstream flow at the mean time of observation of the other three sections suggests lags of 10 to 11 days at the Riverdale section and 20 to 21 days for the Sandy Hook and Governors Island sections. These latter values are approximate at best, because mean times of observations had to be used, but they accord well with the reported delay of 20 days for the adjustment to reach the upper harbor, reported by Ayres (*5*) on the basis of determinations of the fresh water fraction made by salinity measurements.

Additional surveys of the currents and salinity in New York harbor by the U.S. Coast and Geodetic Survey this year should provide data which will delineate and explain the upstream bottom currents more fully and which will make it possible to determine more accurately the role of river discharge and the effects of the East River not considered here.

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Effect of Trypsin Inhibitor on Passage of Insulin Across the Intestinal Barrier

The finding of trypsin inhibitor in colostrum led to the hypothesis that the physiological role of the inhibitor is to protect the antibodies of colostrum from being digested and thus to facilitate their absorption (*1*). Some circumstantial evidence confirming this hypothesis has been accumulated (*2, 3*). For a direct experimental assault, insulin was chosen as the test protein, because its passage into the blood stream is reflected by the blood sugar level.

Early attempts to administer insulin through the gastrointestinal tract have been reviewed by Jensen (*4*). It is interesting to note that Murlin and Hawley (*5*) and Eaton and Murlin (*6*) used blood plasma as a source of "antitrypsin," whereas Harned and Nash (*7*) used an extract from *Ascaris*. The quantities of the inhibitor present in such preparations were, however, much lower than those used now. The maximal positive effect reported was a temporary disappearance of glycosuria in depancreatized dogs, with (*6*) or without a significant (*7*) lowering of the blood sugar level.

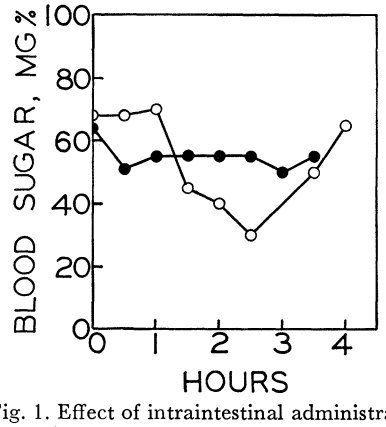

Fig. 1. Effect of intraintestinal administration of insulin on the blood sugar level. Open circles, experiments in which 6 units of insulin (40 units/kg) plus 40 mg of pancreatic inhibitor were injected. Solid circles, control experiments, in which 6 units of insulin (40 units/kg) (no inhibitor) were injected.

A systematic study of different trypsin inhibitors has revealed striking differences with respect to their susceptibility to peptic digestion (*2*) and to their ability to inhibit chymotrypsins (*8*). When these properties were taken into account, only colostrum inhibitor and pancreatic inhibitor were indicated for further study. Pancreatic inhibitor was more easily obtained and thus was used. Once-crystallized inhibitor was prepared according to the method of Kunitz and Northrop (*9*) from "fraction E" (*10*). The regular zinc insulin used was a commercial product (*11*).

Male Sprague-Dawley rats, weighing about 150 g each, were fasted overnight and were anesthetized with Pentothal (thiopental sodium, 40 mg/kg of body weight). The solutions to be investigated were mixed and injected into a loop of jejunum 20 cm long, ligated on both ends. Blood was obtained by clipping off the tip of the tail. Glucose content was determined by the Nelson-Somogyi method (*12*).

Ten experiments in which insulin and inhibitor were injected together were performed. In all ten, a significant drop in blood sugar was observed. Figure 1 illustrates the experiment in which the lowest, and Fig. 2, that in which the highest, dose was used. In other experiments, intermediate doses were used. Ten control experiments were performed by injecting insulin without inhibitor (Figs. 1 and 2); all results were negative. Two control experiments in which the inhibitor alone, and an additional experiment in which insulin plus an excess of protamine, was used, also gave negative results. None of the ten experimental animals died of insulin shock. The highest dose (Fig. 2) produced an effect approximately equivalent to 8 units/kg injected intraperitoneally, suggesting that, at the most, 3 percent of the injected insulin was absorbed.

Substitution of soybean inhibitor for pancreatic inhibitor, in amounts equivalent with respect to trypsin inhibiting power, resulted in very small and nonuniform responses. Since about 80 percent of each inhibitor remained in the loop after 4 hours of exposure, the difference cannot be ascribed to the instability of soybean inhibitor but suggests that pancreatic inhibitor partially protects insulin against destructive agents other than trypsin, whereas soybean inhibitor does not.

It had not yet been established that pancreatic inhibitor protected insulin from destruction. Inactivation in vivo occurred too fast for convenient measurements—that is, in the presence of 40 mg of inhibitor, of 35 units of insulin injected into the loop, only 5 percent was recovered after 3 minutes and less than 1 percent after 30 minutes; the absence of inhibitor did not influence the recovery of insulin after a short exposure, and barely a trace was recovered after 30 minutes. It was decided, therefore, to measure the rate of destruction of insulin in vitro,

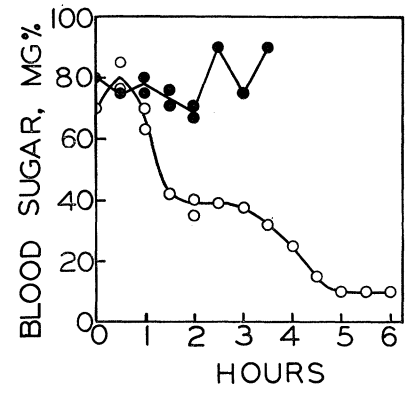

Fig. 2. Effect of intraintestinal administration of insulin on the blood sugar level. Open circles, experiments in which 35 units of insulin (250 units/kg) plus 100 mg of pancreatic inhibitor were injected. Solid circles, control experiments in which 35 units of insulin (250 units/kg) (no inhibitor) were injected.

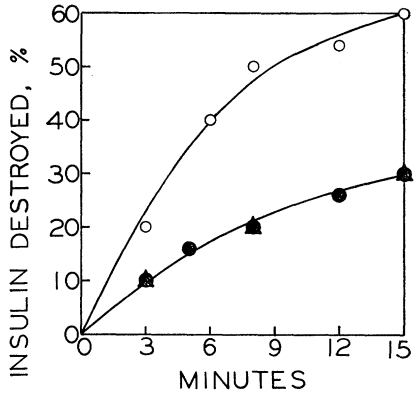

Fig. 3. Rate of destruction of insulin in vitro. Enzymes for the top curve (○) were obtained by injecting into a jejunal loop 1 ml of saline, allowing it to remain 10 minutes, excising the loop, and combining the contents with a 0.5-ml saline washing. Enzymes for the bottom curve (●) were obtained by the same procedure, except that saline containing 40 mg of pancreatic inhibitor per milliliter was used. The incubation mixture consisted of 0.4 ml of enzyme, 2.6 ml of saline containing 0.01*M* phosphate (*p*H 7.3), and 1 ml of insulin, 80 units/ml, at temperature of 37°C. At indicated times aliquots were withdrawn and diluted. In our control experiments, subcutaneous injection of 0.6 units/kg decreased the blood sugar level 35 to 45 percent, when the 1-, 2-, and 3-hour values were averaged and expressed as a percentage of the zero time value. Only dilutions of the in vitro enzyme-insulin mixtures which led to responses in this range were used to calculate percentage of inactivated insulin. Solid triangle, enzymes A to which pancreatic inhibitor was added before the addition of insulin.