Bulletin No. 187 - The Metabolism of Honeybees in Winter Cluster

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The Metabolism of Honeybees
in Winter Cluster

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The Metabolism of Honeybees in Winter Cluster

By

C. L. Corkins and C. S. Gilbert

Introduction

Honeybees, like other insects, are essentially cold-blooded animals. However, in their colonial life, honeybees do possess the power of regulation of temperature to a limited extent. A high summer temperature causes them to circulate air through the hive by fanning. This process rapidly evaporates water within the colony and results in the cooling of the air. Such regulation occurs when the temperature is above 36.1° C. (97° F.). Adjustment of temperature is also accomplished by clustering. Partial clustering protects the brood from cold weather during early spring and late summer. Clustering, however, is more commonly a winter phenomenon. The winter cluster is formed at a temperature of approximately 13.9° C. (57° F.).

The winter cluster is primarily a device for the conservation of the heat produced by metabolism. Its efficiency in this regard is of a very high order. Moreover, this efficiency is capable of adjustment within certain limits, according to need, by contraction or expansion, causing the tightening or loosening of the cluster.

A study of the heat production of honeybees in winter has been made by Milner and Demuth (1). The carbon dioxide output of a cluster of bees was determined by means of a respiration calorimeter. However, there were no means of changing the temperature within the chamber which contained the bees. During the ten days of the experiment the temperature of the air in the chamber varied from 6.1° C. to 9.2° C. Hence there was no opportunity to correlate metabolism with varying degrees of temperature. A colony of 9,635 bees under these conditions produced 129.9 liters of carbon dioxide, or 688 calories of heat, in ten days. This is approximately 0.1 calorie per gram of bees per day. Such

*In this study Mr. Gilbert has been responsible for the development of the method of determining the carbon dioxide. The section of the bulletin dealing with the technique of carbon dioxide determination was written by Mr. Gilbert.
a metabolism was similar to the output of 7,000 calories per day by a man weighing 154 pounds, which is found only in the case of exceedingly hard, manual labor.

Farrar (2) studied the metabolism of a small number of adult honeybees in an artificial environment throughout the thermal life range by means of a micro-respirometer. At a temperature of 0.3°C the bees became inactive, and the metabolic rate was very low. Such bees recovered after an exposure to this temperature of 24 hours or more. For the second hour of exposure at this temperature, the oxygen consumption was 29.6 cubic millimeters per hour per bee. At 14.0°C the corresponding oxygen consumption was 265.2 cubic millimeters. For the most part, there was a positive correlation of metabolic rate with temperature throughout the range of life. Farrar states that "there may be a temperature between 0.3°C and 14°C at which a basal condition might be approached, but such a temperature was not determined in these experiments."

Studies (3) of the metabolism of colonies of bees wintered out of doors indicated that there was no increase of activity as outside temperatures dropped below the clustering temperature. The exact metabolic rate under different temperature conditions could not be ascertained by the methods used. For this reason, it seemed desirable to conduct an experiment in which the temperature could be controlled and the metabolism of the colony measured with precision. The methods used and the results obtained with such an experiment are given herewith.

As a theoretical basis for practice in the wintering of bees, it is desirable to determine the temperature conditions of basal metabolism of the colony. The colony as a whole, and not individuals, must be considered. The temperature which produces the basal metabolism of the colony may be considered as that temperature at which the rate of metabolism is at a minimum with the individual life processes imperative to existence still going on. Since it is practically certain that the temperature at which such a rate of metabolism takes place is below the clustering temperature, a condition peculiar to the clustered bees is introduced. The process of clustering pulls the colony away from its food supply.
If the colony is held in a tightly formed cluster over an extended period of time, the food supply must be replenished or else the bees will perish of starvation. The temperature must not be so low that the bees either cannot move the entire cluster upward or break away from it in order to secure additional food. It is possible that the minimum rate of metabolism may be at a temperature so low that the bees cannot break from or move the cluster. Still the colony may live normally for many days under such a condition. Hence the term “basal metabolism,” as hereafter applied to the colony of bees, means the minimum normal metabolism at which life can be maintained over an indefinite period of time. The temperature which produces basal metabolism will permit, therefore, the replenishment of the cluster with food when necessary.

Temperature Control.

The apparatus selected for variation and control of the temperature of the compartment in which the bees were placed is commonly known as the Frigidaire ice cream cabinet. This cabinet is cooled by brine which makes possible a temperature of \(-10^\circ\) to \(-15^\circ\) F. when needed. Furthermore, the temperature can be controlled within a range of about \(2^\circ\) F. Any changes in temperature are very gradual because of the large body of brine. The compartment is \(20'' \times 20'' \times 22''\) in size, which makes it large enough to receive a full colony of bees in a standard hive body. It is metal lined and air tight. A special lid was constructed with two conduit holes, but otherwise solid. In use, this lid is hermetically sealed to the top of the compartment.

Determination of Temperatures.

Temperatures were taken by the thermocouple method. Forty-two thermocouples were placed at various levels in the hive and between the different frames. It was impossible for the bees to cluster without covering several of the thermocouples. Two thermocouples were mounted in the air outside the hive, but within the compartment. Temperature readings of all 44 thermocouples were taken hourly.
In order to obtain a mean cabinet temperature, the readings of the two couples outside the hive were averaged. The mean hive temperature was computed from an average of the five couples in the bee-free space of the hive which gave the lowest readings. The mean cluster temperature is an average of the five highest temperatures within the cluster. The mean maximum cluster temperature is the mean temperature of the so-called "hot spot," that is the thermocouple giving the highest reading in the cluster. In the cases where these mean temperatures are given for periods of considerable duration and are accompanied with their probable errors, they were computed from a frequency table distribution.

PRINCIPLES OF METHOD USED FOR GAS ANALYSIS

In the great majority of cases the analysis of air for carbon dioxide content in metabolism experiments has been made by chemical methods. After preliminary trials using one of these methods, it was decided to change to a physical method as being less expensive in the long run, giving rapid and analytical results almost as accurate as the chemical method, and requiring a great deal less labor. Then, too, the physical method had the great advantage of being readily changed, if desired, to a self-recording system which would show continuous analyses of the gas as it passes through the system.

The thermal conductivity method of gas analysis is extremely simple in principle. If a heated wire is surrounded by a gas composed of air and carbon dioxide, the rate of flow of heat from the wire through the gas will depend on the percentage of carbon dioxide in the gas, other factors such as pressure and surrounding temperature being constant. Another way of stating the same thing is to say that carbon dioxide-free air, and air containing carbon dioxide, have different thermal conductivities. These differences are small but are readily measurable. Thus carbon dioxide-free air has a thermal conductivity at 100° C. of 0.0000719, and air containing 20% CO₂ of 0.0000674 (4).
If, then, there are two wires initially heated to the same temperature and so insulated that heat losses by radiation and convection are made very small, but the one wire is surrounded by carbon dioxide-free air while the other is surrounded by air containing a certain per cent of carbon dioxide, it is evident that the former will cool off more rapidly than the latter, since the medium surrounding the former is a better conductor of heat. If an equal amount of heat is continually supplied to each wire in a short time each will come to a constant temperature, the wire in the carbon-dioxide-free air being cooler than the other, the difference in temperature between the two wires depending on the amount of carbon dioxide in the medium surrounding the warmer wire. Consequently, some means of determining the difference in temperature, and a knowledge of the amount of carbon dioxide such a difference corresponds to, is all that is necessary in the analysis of the air containing carbon dioxide.

Description of Apparatus. The difference in temperature may be determined with the aid of a Wheatstone bridge arrangement such as is shown diagramatically in Figure 1. Here advantage is taken of the fact that the temperature coefficient of electrical resistance of platinum is comparatively high. A 12-volt storage battery (S.B.) furnished a small constant current regulated by a
variable resistance \( (R_1) \) which heats the platinum wires in the bridge. Two platinum resistance wires \( (W_1 \text{ and } W_2) \) with the resistances \( R_3 \) and \( R_4 \), and the slide wire resistance \( R_2 \), form the Wheatstone bridge proper. The bridge is balanced by varying \( R_2 \), while the platinum wires are surrounded by dry carbon dioxide-free air, the position of balance being shown by lack of deflection in the galvanometer \( G \), since, when balanced, the current is equally divided between the branches \( W_1 R_4 \) and \( W_2 R_3 \). If air containing carbon dioxide surrounds one of the wires \( (\text{as, for instance, } W_2) \), that wire becomes warmer, its electrical resistance increases, and the bridge will become unbalanced because less current will flow through \( W_2 \) and \( R_3 \) than through \( W_1 \) and \( R_4 \) since the resistance of the latter pair is unchanged while the resistance of \( W_2 \) has increased. The change in resistance in the wire is proportional to the change in temperature which, in turn, is a function of the amount of carbon dioxide in the air surrounding the wire.
The thermal conductivity cell used in the metabolism work was one made by Charles Englehard, Newark, N. J., and is shown photographically in Figure 2 and diagrammatically in Figure 3. The details of its construction are given by W. F. Hamilton (5). In brief, it consists of two platinum spirals embedded in quartz, corresponding to W₁ and W₂ of Figure 1, with ratio coils and slide wire in the bottom of the cell corresponding to R₃, R₄ and R₂ and connected as shown in Figure 1. It should be observed that any gas reaching either of the spirals comes in contact with that spiral only by means of a by-pass from the main flow of gas, and, also that one of the spirals (No. 1 of Figure 3) is hermetically sealed in an atmosphere of dry air.

In order to know the weight of carbon dioxide given off by the bees it is necessary to know the volume, temperature, pressure, and per cent of carbon dioxide in the air drawn from the compartment containing the bees. To obtain this information the
The metabolism of honeybees

The set-up shown in Figure 4 was devised. The electrical connections are shown in Figure 5. Air is drawn through the soda lime towers which remove the carbon dioxide from it, and then the air passes into the metabolism compartment in the low temperature cabinet. This hermetically sealed compartment contains the colony of bees, the compartment temperature being regulated by the cabinet. From here the air, now containing carbon dioxide and moisture from the bees, is drawn past a thermocouple for temperature determination and then past a side arm attached to a mercury manometer so that the pressure may be determined at this point, which practically means the pressure in the metabolism cabinet. The air is next drawn through about twenty feet of copper tubing placed in an oven maintained at a constant temperature of 29°-31° C. so as to warm the air from the cold compartment up to approximately room temperature, and from here the air goes through two towers filled with calcium chloride to remove the moisture. The air current is then divided, part going through the stopcock S₁ past a manometer side arm, another thermocouple, and then into the thermal conductivity cell, which is kept at a constant temperature of 30° C. in an oven. Of course, here the percentage of carbon dioxide is determined. From the cell the air passes into a bottle containing glycerin and is then united with the remainder of the air which has not passed through the cell. The rate of bubbling through the glycerin gives an approximate idea of the rate of air flow through the cell, while the by-pass through S₂ makes possible considerable variation in the flow of air through the conductivity cell. The air is next drawn through another calcium chloride tower which serves to prevent moisture reaching the thermal conductivity cell from this side of the system, and then through water to saturate the air, and, finally, through a precision gas meter which reads to 0.001 of a cubic foot. Since the gas meter is partially filled with water and its accuracy partially depends on keeping this water at a certain level, it is necessary to provide some means of preventing the air which passes through it from evaporating off any considerable amount of this water. A humidifier is thus necessary for the air which has been thoroughly dried previously. The stopcocks at the end of the system serve as means
Figure 5. Wiring Diagram of Metabolism Apparatus.
of regulating for the whole system the amount of suction which is maintained by a motor-driven suction pump. Thus the gas meter, with its manometer and thermometer, gives the volume, pressure and temperature of the air, while the thermal conductivity cell shows its composition.

In Figure 5 it will be noted that by means of the lamp bank connected across the storage battery circuit it is possible to obtain the approximate voltage of the storage batteries and hence determine their constancy and whether in a charged or discharged condition. The diagram also shows both a gas analysis recorder and a potentiometer connected to the thermal conductivity cell. As a matter of fact, all results up to 1932 have been obtained by the use of either the potentiometer, as illustrated, or a millivoltmeter, but it is expected that for future work the automatic recorder will be used.

When the millivoltmeter or potentiometer is connected to the galvanometer leads (G+ and G− of Figure 3) of the thermal conductivity cell containing the heated platinum wires, these instruments show a certain voltage, depending on the composition of the gas being passed through the one side of the cell. To ascertain the percentage of carbon dioxide from voltage readings, it is necessary, first, to balance the cell when dry air surrounds both spirals, and then to pass air containing different amounts of carbon dioxide through one side, noting the voltage so obtained, and by chemical analysis determining at the same time the corresponding percentage of carbon dioxide. From these data a calibration curve can be drawn, in this case a straight line, by which, the voltage being known, the corresponding percentage of carbon dioxide can be interpolated.

Calibration of Thermal Conductivity Cell. The apparatus shown in Figure 4 was set up, tested for leaks, and then dry carbon dioxide-free air was drawn through the thermal conductivity cell simply by eliminating the metabolism compartment from the gas flow. The storage batteries were connected through the rheostat to the cell and the rheostat adjusted until the ammeter showed a constant current of 0.240 amperes. If the heat dissipation from
Figure 6. Apparatus for Calibrating Thermal Conductivity Cell.
each spiral had been the same, then no voltage would have shown in the galvanometer leads of the cell since the same gas (dry air) now surrounded both spirals. However, since there was actually a reading of about "negative" 0.51 millivolts (if the voltage produced by air containing carbon dioxide is considered "positive") the adjusting screw (see Figure 3) was turned until the voltage was "negative" 0.270 millivolts. Further adjustment was not considered necessary.

The problem of securing a gas mixture of carbon dioxide and air in which the ratio remained constant for a considerable length of time (two hours or more), but in which the ratio could be changed as desired, proved to be a vexing one. It was not until the apparatus was set up, as shown in Figure 6, that any satisfactory results could be obtained. Bottle B, though open to the air, was kept filled with CO$_2$ by a rapid flow of gas from the carbon dioxide pressure tank through the Bottle A which was partially filled with water. By suction from the main system CO$_2$ was slowly drawn from B and through C, which served as a bubble

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**Figure 7. Calibration Graph of Thermal Conductivity Cell.**

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counter, and then mixed with air which was at the same time sucked through D. E served as a mixing jar, and the calcium chloride tower removed a large part of the moisture before the gas entered the main system. By means of the stopcocks S₁ and S₂ and the bubble counters C and D, which were partially filled with water, it was possible to obtain any variation in ratio desired between air and carbon dioxide.

A gas sampling tube was inserted in the main system between the warming oven and the calcium chloride towers, and when the voltage of the cell came to constant value the sampling tube was removed and the gas sample analyzed chemically for CO₂. This procedure was repeated with various ratios of air and CO₂, and by plotting on graph paper the results thus obtained the calibration chart for the cell (Figure 7) was made. It can readily be seen that the results fall practically on a straight line, experimental error probably accounting for the variations.

General Discussion. The voltages obtained from the thermal conductivity cell were fairly independent, within the range tested, of the rate of gas flow. The rate used in the calibration and subsequent experiments was 1.0 (±0.3) cu. ft. per hour. When the rate was decreased to 0.28 cu. ft. per hour, the voltage changed by 0.05 millivolts, corresponding to about 0.1 per cent CO₂, while when the rate was increased to 2.5 cu. ft. per hour, no change in voltage reading from the reading at 1.0 cu. ft. could be detected.

Since the storage batteries were being constantly charged from the alternating current line, they furnished a steady current to the cell over long periods of time. In actual practice it has been found necessary to increase or decrease the resistance by the rheostat slightly so as to maintain a constant amperage of 240 milliamperes, the current recommended by the makers of the cell. The variation in current was sufficient to cause the ammeter needle once in a while to be just slightly to one side or the other of the line marking 240 milliamperes, a variation perhaps of 1 to 2 milliamperes. A change of this amount did not appreciably change the voltage of the cell.
When used commercially, the thermal conductivity cell is not insulated from changes in room temperature. In this work, however, it was desired to obtain as accurate results as possible, and, since constant and reproducible readings could not be obtained with the cell exposed to fluctuations in room temperature, it was mounted in a constant temperature oven where the variation was not over 0.5° C. When exposed to room temperatures with maximum variation of 5° C., the cell voltage varied 0.29 millivolts, corresponding to about 0.5 per cent CO₂; when the cell was in the oven the maximum variation in voltage was 0.02 millivolts, corresponding to about 0.04 per cent CO₂. Sudden changes in temperature must have considerable effect on the voltage, for raising the temperature of the oven slowly to a temperature 6° C. higher than previously caused a change in voltage of only 0.13 millivolts. During the experiments on metabolism the oven temperature was maintained at 30° ±0.5° C.

After the whole apparatus was set up and calibrated, it required very little attention except for the taking of readings which were recorded every 15 minutes. It possesses the added advantages of indicating the carbon dioxide content of the air at any particular time besides the total amount of carbon dioxide given off. Thus the activities of the bees can at all times be closely followed.

GENERAL PROCEDURE AND DATA OBTAINED

Organization of the Experiments. Three separate experiments with three different colonies of bees were conducted. For purposes of differentiation, these experiments are designated as series 1, 2, and 3, given in the order in which they were made.

The colony in each separate experiment of series 1 and 3 was subjected to several different temperature conditions for an arbitrary length of time. The first period to which a colony was subjected to a certain fixed range of temperature is designated as chronological period 1. At the close of such a period, the temperature was changed to another fixed range, either several degrees higher or lower, and this second stage of the experiment is known as chronological period 2. Other chronological periods follow ac-
cording to the number of times the temperature conditions were changed. As will later be seen, the temperature range for each period is very narrow, being held to the minimum attainable with the apparatus. In fact, the temperature of the cabinet during each chronological period is better described as constant rather than a narrow range.

In series 1 and 3 the term "check-in period" is used to indicate the duplication of previous temperature conditions in the same experiment.

No chronological periods are designated in series 2 as the temperature of the cabinet was not changed throughout the experiment.

Relative Humidity. No effort was made to control the relative humidity in the cabinet. However, the general relative humidity conditions were ascertained. This was accomplished by placing a Friez seven-day hygro-thermograph in the cabinet on top of the hive at the beginning of the experiments. This instrument ran with one winding of the clock for ten days, giving a chronological history of the relative humidity for that time. When the clock stopped a record of only the maximum and minimum relative humidity was secured, as the instrument was sealed inside the cabinet.

During the first ten days of series 1, the relative humidity varied from 68 per cent to 86 per cent, with a mean of 80 per cent. For the final 10 days of the 20 days of the experiment, the minimum and maximum relative humidities were 72 per cent and 88 per cent. The hygro-thermograph failed to function during series 2, and no records are available.

During series 3, chronological period 1 (see Table 3), the relative humidity was very high. It ran from 86 per cent to 100 per cent. During chronological period 2, when the temperature was lowered, the relative humidity varied from 78 to 85 per cent. After the clock stopped, the minimum and maximum relative humidities recorded were 71 per cent and 100 per cent.
In general, it may, therefore, be said that the relative humidity in the cabinet was very high. This was entirely due to the release of metabolic water by the bees. The air drawn into the cabinet was taken from the laboratory in which the experiment was conducted. The relative humidity in this room is unusually low, being about 18 per cent to 20 per cent.

The Bees. The bees used in this experiment were Caucasians of the Rauchfuss strain. This strain has been selectively bred under intermountain conditions for about 35 years from stock originally imported from the Caucasian Mountains of Europe. During this time there has been a slight infiltration of Italian stock into these Caucasians. For the most part, however, the race has been kept remarkably pure. Comparative studies between Caucasians and Italians were not attempted in this experiment.

With one exception, the bees were taken out of winter quarters for the experiment. Prior to placement in the cabinet they were wintered in the open without packing. In each case the transfers were made after warm weather had allowed for general cleansing flights. The bees were transferred during such a period of warm weather to an eight-frame observation hive. The paneled sides of this hive were removed when the colony was placed in the cabinet. Screen wire over these openings confined the bees but allowed free movement of the air and rapid adjustment of the hive temperature to the cabinet temperature. To further facilitate the rapid drainage of carbon dioxide out of the hive into the cabinet, holes were drilled in the bottom of the hive and covered with screen wire. The entrance to the hive was plugged during the experiment. The first temperature run of the cabinet was always low enough so that the bees would quickly settle down and form a cluster upon their introduction into it.

At the beginning of each experiment care was taken to see that only live bees were in the hive. Then at the close of the run the bees were killed by the introduction of calcium cyanide. This facilitated the actual count of every bee that was in the hive.
TABLE I.
SUMMARY OF TEMPERATURE AND CARBON DIOXIDE DATA
March 6-26, 1931

<table>
<thead>
<tr>
<th>Chronological Order of Periods</th>
<th>Duration in Hours</th>
<th>Mean Cabinet Temperature °C.</th>
<th>Mean Hive Temperature °C.</th>
<th>Mean Cluster Temperature °C.</th>
<th>Mean Maximum Cluster Temperature °C.</th>
<th>Grams CO₂ Per Hour</th>
<th>Milligrams CO₂ Per Hour Per Bee</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>163</td>
<td>13.82±.07</td>
<td>17.56±.08</td>
<td>31.71±.03</td>
<td>33.48±.01</td>
<td>3.6034</td>
<td>0.288</td>
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<td>82</td>
<td>13.32±.08</td>
<td>15.41±.07</td>
<td>28.31±.05</td>
<td>32.62±.03</td>
<td>2.5936</td>
<td>0.207</td>
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<td>2</td>
<td>155</td>
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<td>4.34±.02</td>
<td>28.73±.05</td>
<td>32.57±.03</td>
<td>1.8961</td>
<td>0.151</td>
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<tr>
<td>4</td>
<td>91</td>
<td>2.82±.02</td>
<td>4.21±.02</td>
<td>24.22±.10</td>
<td>28.95±.09</td>
<td>1.6090</td>
<td>0.128</td>
</tr>
</tbody>
</table>

DATA ON SERIES NUMBER I

Since the minimum rate of metabolism has previously been considered to be at or near the clustering temperature, the first temperature selected for study was that just sufficiently cold to hold the bees either in cluster or inactive on the combs. The mean cabinet temperature was 13.8°C., and the mean hive temperature was 17.5°C. At the close of the above period 1, the cabinet was then set to run at about 3°C. above the minimum critical temperature for life of the individual bee, a temperature presumed to produce rapid metabolism by induced activity and, therefore very bad for the bees. The mean temperature of the cabinet for this second run was 2.8°C., which produced a mean hive temperature of 4.3°C. The rate of metabolism was again determined at each temperature of the two initial runs with check-in periods 3 and 4. Thus, if the low temperature had resulted in the death of a large number of bees, and the metabolism of the colony as a whole reduced thereby, it would immediately be apparent when the higher temperature run was checked.

There were 12,483 bees in the colony used in this experiment. The results obtained during series 1 are given in Table I, which also includes the essential temperature data. A very decided decrease in the rate of metabolism is shown for both of the colder temperatures as contrasted with the temperatures at or near clustering. In the case of the original runs, the rate of metabolism...
for the cold period is 52.6 per cent of that of the warm period. For the check-in periods, the ratio is 62.0 per cent.

A minor tendency is also noted. There seems to be some relationship between the rate of metabolism and length of time of confinement. In the case of both temperatures, the check-in periods show a lower rate of metabolism than the primary runs. The temperatures in these two instances were so similar that these differences can scarcely be laid thereto. Examination of the colony at the close of the experiment indicated that the loss of bees with the progress of time probably effected the rate of metabolism but very little.

**DATA ON SERIES NUMBER 2**

This experiment was set up with the idea of checking the possible relationship between the rate of metabolism at a given temperature with progression of time. The temperature was held

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**TABLE II.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Mean Cabinet Temperature °C</th>
<th>Mean Hive Temperature °C</th>
<th>Mean Maximum Cluster Temperature °C</th>
<th>CO₂ Per Hour Grams</th>
<th>CO₂ Per Hour Per Bee Milligrams</th>
</tr>
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<tr>
<td>April</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>6</td>
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<td>0.135</td>
</tr>
<tr>
<td>11</td>
<td>5.80</td>
<td>8.22</td>
<td>31.57</td>
<td>2.3010</td>
<td>0.129</td>
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<tr>
<td>12</td>
<td>5.74</td>
<td>8.00</td>
<td>30.59</td>
<td>2.6977</td>
<td>0.163</td>
</tr>
<tr>
<td>13</td>
<td>5.60</td>
<td>8.04</td>
<td>32.09</td>
<td>2.4907</td>
<td>0.150</td>
</tr>
<tr>
<td>14</td>
<td>5.48</td>
<td>7.87</td>
<td>31.94</td>
<td>1.9314</td>
<td>0.115</td>
</tr>
<tr>
<td>15</td>
<td>5.83</td>
<td>8.18</td>
<td>32.50</td>
<td>1.8342</td>
<td>0.111</td>
</tr>
<tr>
<td>16</td>
<td>5.81</td>
<td>8.44</td>
<td>32.83</td>
<td>1.7661</td>
<td>0.107</td>
</tr>
<tr>
<td>17</td>
<td>5.68</td>
<td>8.30</td>
<td>32.53</td>
<td>1.8430</td>
<td>0.111</td>
</tr>
<tr>
<td>18</td>
<td>5.82</td>
<td>9.08</td>
<td>32.72</td>
<td>1.9257</td>
<td>0.116</td>
</tr>
<tr>
<td>19</td>
<td>5.70</td>
<td>9.07</td>
<td>32.45</td>
<td>2.0795</td>
<td>0.126</td>
</tr>
</tbody>
</table>
as constant as possible, the maximum amplitude of variation in the cabinet during the 14 days was 1.9° C. After the cluster had definitely formed and settled, the variation in the hive temperature was even less than this.

There were 16,500 bees in this cluster.

The data on this experiment by 24-hour periods are given in Table II. In view of the fact that the experiment was conducted for only 14 days, it leaves unanswered the question of the effect of confinement over a long period of time in such a tightly formed cluster as was produced by a hive temperature of about 8 to 9° C. There is a gradual reduction in the rate of metabolism over this short period. It is particularly apparent, however, only during the first three days. After this time the change from day to day is not particularly significant. In fact, the carbon dioxide output for the last six days is remarkably constant. It would appear that bees gradually settle into a more or less inactive state after the cluster forms. During this settling process, there is a comparatively high rate of metabolism. This situation is probably attendant with every making and breaking of the cluster under natural outdoor conditions.

At the close of the experiment the colony was in a normal condition.

**DATA ON SERIES NUMBER 3**

The age condition of the bees in this series differs from that of either of the others. Owing to a break in the equipment, it was impossible to conduct another experiment until June 6. As a consequence, the bees were largely young, following at least two good cycles of brood rearing. Before placing these bees in a cabinet, they were shaken into a combless package and the queen was caged. When they were transferred to combs in the observation hive, to be placed in the cabinet, the queen was still confined. Upon introduction into the cabinet, the temperature was 7.5° C., which caused immediate clustering. The queen was then released. Examination of the combs at the close of the experiment showed that no brood rearing had taken place.

There were 11,998 bees in this colony.
March, 1932 The Metabolism of Honeybees

TABLE III.
SUMMARY OF TEMPERATURE AND CARBON DIOXIDE DATA
June 6—July 30, 1931.

<table>
<thead>
<tr>
<th>Chronological Order of Periods</th>
<th>Duration in Hours</th>
<th>Mean Cabinet Temperature °C.</th>
<th>Mean Hive Temperature °C.</th>
<th>Mean Cluster Temperature °C.</th>
<th>Mean Maximum Cluster Temperature °C.</th>
<th>Grams CO₂ Per Hour.</th>
<th>Milligrams CO₂ Per Bee</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>48</td>
<td>14.80±.10</td>
<td>18.80±.29</td>
<td>29.33±.05</td>
<td>30.00±.05</td>
<td>6.2199</td>
<td>0.518</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>14.25±.15</td>
<td>19.63±.19</td>
<td>28.93±.06</td>
<td>29.54±.07</td>
<td>6.7605</td>
<td>0.563</td>
</tr>
<tr>
<td>1</td>
<td>120</td>
<td>7.51±.01</td>
<td>9.35±.11</td>
<td>29.26±.06</td>
<td>29.84±.04</td>
<td>7.2351</td>
<td>0.604</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>7.27±.09</td>
<td>9.71±.09</td>
<td>29.35±.02</td>
<td>30.33±.05</td>
<td>4.3309</td>
<td>0.360</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>0.27±.03</td>
<td>0.71±.05</td>
<td>29.27±.04</td>
<td>29.75±.03</td>
<td>6.4408</td>
<td>0.536</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>0.03±.10</td>
<td>0.14±.04</td>
<td>28.56±.06</td>
<td>29.80±.06</td>
<td>3.7625</td>
<td>0.313</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>-6.52±.07</td>
<td>-5.02±.09</td>
<td>28.63±.04</td>
<td>29.25±.02</td>
<td>6.0248</td>
<td>0.502</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>-6.25±.09</td>
<td>-4.17±.09</td>
<td>28.68±.03</td>
<td>29.27±.04</td>
<td>5.7236</td>
<td>0.477</td>
</tr>
</tbody>
</table>

The data for this series are given in Table III. Three of the cabinet temperatures were generally similar to those in series 1. A still lower temperature of approximately —6° C. was also used. There seem to be certain inconsistencies in the output of carbon dioxide over the various temperature ranges. One wonders if the age of the bees had anything to do with this, as all other conditions were the same as in the previous experiment. It is possible that the chronology of the runs in part explains some of these inconsistencies. It was natural to expect that the initial run would have a high rate of metabolism, and the check-in period on this temperature (7° C.) shows the rate decreased 40.3 per cent. There is a similar decrease in the check-in period at 0° C. The fact that the rate of metabolism is somewhat lower at 14° C. than might have been expected after an examination of the data for series 1 may partially be explained by the fact that this temperature was used after the bees had been subjected for 72 hours to a temperature of —6.5° C. However, it is doubtful if enough bees were killed during this comparatively cold period to greatly affect the carbon dioxide output.
Without fully understanding definitely just what took place, it can be safely said that there was no increase in the rate of metabolism as the temperatures were lowered below the clustering temperature, and the tendency was toward reduction.

One very significant fact is apparent. The rate of metabolism per bee in this series is much greater than in either of the previous experiments. This raises two closely correlated questions which, as yet, cannot be answered. The first is: Do bees have to attain some certain age before they withstand sub-clustering temperatures efficiently? The second is: Do bees pass through a hardening process during the fall which affects their rate of metabolism at a given temperature?

**Carbon Dioxide Tolerance.** At the close of series number 2, a brief study of carbon dioxide tolerance was made. At 2:00 p.m., April 20, the flow of air through the cabinet was stopped. The cabinet was kept closed for 24 hours. The percentage of carbon dioxide coming from the cabinet at the beginning of this period was 4.21. At irregular intervals, gas was drawn from the cabinet five times during the 24-hour period in order to determine the carbon dioxide content. The percentages and temperatures at these intervals were as shown in Table IV.

<table>
<thead>
<tr>
<th>Time</th>
<th>Cabinet Temperature °C</th>
<th>Mean Cluster Temperature °C</th>
<th>Maximum Cluster Temperature °C</th>
<th>Per Cent CO₂ in Cabinet</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:00 p.m.</td>
<td>5.5</td>
<td>30.9</td>
<td>31.8</td>
<td>4.21</td>
</tr>
<tr>
<td>4:00 p.m.</td>
<td>5.9</td>
<td>30.9</td>
<td>31.9</td>
<td>7.39</td>
</tr>
<tr>
<td>8:00 p.m.</td>
<td>6.0</td>
<td>30.2</td>
<td>31.6</td>
<td>12.38</td>
</tr>
<tr>
<td>1:30 a.m.</td>
<td>5.8</td>
<td>26.0</td>
<td>28.3</td>
<td>17.67</td>
</tr>
<tr>
<td>8:00 a.m.</td>
<td>5.3</td>
<td>16.8</td>
<td>17.8</td>
<td>21.30</td>
</tr>
<tr>
<td>2:00 p.m.</td>
<td>5.4</td>
<td>13.3</td>
<td>14.9</td>
<td>21.09</td>
</tr>
</tbody>
</table>

At 2 p.m., April 21, there had been no increase in the carbon dioxide content of the gases in the cabinet over the previous six-hour period. The mean cluster temperature had dropped from 30.9° C. to 13.3° C. in 24 hours. It seemed likely that most of
the bees, if not all of them, were dead. To determine this point, circulation of the air through the cabinet at the usual rate was started. Within one hour, the mean cluster temperature had jumped to 32.8° C. Twenty-four hours later both the temperatures and the carbon dioxide output had returned to normal. The mean cluster temperature was 30.7° C., and the percentage of carbon dioxide was 4.25, practically the same as at the time of the closing of the air line. When the colony was examined, there was no indication that the bees had suffered from the accumulation of carbon dioxide in the cabinet.

Work Units. The energy expended by bees translated into calories is of interest, although not of any great practical importance. Milner and Demuth (1) compared the work done by bees in calories with that of a working man of average size (154 pounds) as a means of showing the tremendous amount of energy expended under quiescent conditions. On this basis they showed that 154 pounds of bees would expend about 7,000 calories per day, which is an unusual output for a man. Such a comparison, however, involves approximately three-quarters of a million individuals in the case of the bees and only one individual in the case of the average man. A more complete concept is developed when the energy output and honey consumption per individual in the bee cluster is considered.

A summary of the work factors in the honeybee cluster is given in Table V. Only the minimum and maximum rates of metabolism for a 24-hour period have been computed. The temperatures for these periods may be ascertained, if desired, from Tables I, II, and III. In figuring the milligrams of honey used per bee per day, it was necessary to make the computation from the carbon dioxide output. Therefore, the honey consumed is theoretically based on an assumption of a 20 per cent water content and 80 per cent pure C₆H₁₂O₆.

In only two cases was the energy output on the basis of a 154-pound man found to be less than 7,000 calories per day. In one case it was more than four times that amount. From this comparison it would seem that the rate of metabolism is fairly large, even though basal metabolism has probably been closely approached.
TABLE V.
WORK FACTORS OF THE HONEYBEE CLUSTER

<table>
<thead>
<tr>
<th>Series Number</th>
<th>Chronological Period Number or Day</th>
<th>Energy Output in Large Calories per Colony</th>
<th>Energy Output if Weight of Nectar (average man) in Large Calories per Day</th>
<th>Energy Output per Bee in Small Calories</th>
<th>Honey Output (water) per Bee per Day in Milligrams</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>101.32</td>
<td>6,231</td>
<td>8.11</td>
<td>2.636</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>226.91</td>
<td>14,000</td>
<td>14.79</td>
<td>5.903</td>
</tr>
<tr>
<td>2</td>
<td>4-16</td>
<td>111.21</td>
<td>5,190</td>
<td>6.74</td>
<td>2.189</td>
</tr>
<tr>
<td>2</td>
<td>4-6</td>
<td>703.42</td>
<td>18,359</td>
<td>23.84</td>
<td>7.744</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>244.01</td>
<td>15,677</td>
<td>20.33</td>
<td>6.606</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>456.75</td>
<td>29,345</td>
<td>38.07</td>
<td>12.365</td>
</tr>
</tbody>
</table>

A more or less practical question is here involved. In the formation of a tight cluster, the bees pull away from their stores of honey. They may be held in such a cluster under natural conditions for two or three weeks at a time. Cases of survival of clusters of bees under such conditions have been noted (3). Is it possible for bees to carry sufficient honey into the cluster in the pro-ventriculus to tide them over such an emergency?

The maximum load of syrup carried by a honeybee has been observed by Betts (6) to be 90 milligrams. Merrill (7) found that the usual field load of nectar carried is from 18 to 22 milligrams. Parker (8) noted a field load of nectar weighing 24.4 milligrams. The average load of honey carried into the winter cluster is unknown. However, if it were conservatively estimated at 25 milligrams, during the day of April 16 in Series 2, when the rate of metabolism was the minimum for all observations, this supply would last only 11.4 days. In one case it would have lasted only two days. For the average conditions during these experiments, such a supply would last about four days. However, if the bees fully engorged themselves with honey before forming the cluster, these figures should be increased about three-fold. In either event, such a supply is inadequate for many conditions encountered in cold climates.
It is altogether probable that small supplies of stores are placed in the empty cells within the cluster during its formation. During the fall of 1930 an attempt was made to force bees to cluster on wire mesh placed in four frames in the center of the hive. Stores of honey were available on either side. The bees built combs having shallow cells on the screen mesh, and small quantities of honey were transferred to these cells. This was done during weather which produced cluster formation every night.

Activity in the stocking of the cluster with emergency stores seems to be indicated in the metabolism studies. There is a comparatively high rate of metabolism during cluster formation. This higher rate of metabolism may last for a day or more and then gradually lowers to a constant. This is particularly demonstrated in Series 2, although apparent during cluster formation in the other series as well.

Rate of Metabolism in Relation to Temperature. It has previously been held by most authorities on the winter activity of bees that there is a temperature regulation besides the mere conservation of the heat produced by metabolism by means of the cluster. It has been assumed that a declining external temperature causes an increase of activity and a higher rate of metabolism within the cluster, being manifested by the fanning of the wings and the movement of the legs and body. Such activity was thought to progress in intensity with the progress of the decline of the external temperature. The food used to furnish energy for this activity is honey. The metabolism is, as a consequence, primarily carbohydrate, the by-products of which are heat, water vapor, and carbon dioxide.

The data here presented do not support such a theory. On the contrary, the tendency is towards a progressive lowering of activity and the rate of metabolism with the decline of temperature below the clustering temperature. The temperature of the air surrounding the bees which produces basal metabolism is not clearly defined by these data. However, it is apparently between the minimum thermal death point of individual bees (about $-1.0^\circ$ C.) and the clustering temperature (about $13.9^\circ$ C.), and seems to lie within a temperature range of about $4^\circ$ to $8^\circ$ C.
Rate of Metabolism in Relation to Wintering Practice. The activity of honeybees in the cluster in relation to temperature has been used as a theoretical basis in the practice of the wintering of bees. In the wintering of bees outdoors in cold climates, protection, such as the use of expensive packing cases permitting varying degrees of insulation, has been advised in order to prevent induced activity at sub-clustering temperatures. It has been held to be desirable to keep the hive temperature at or somewhat above the clustering temperature.

These data indicate that such practices are in error. The beekeeper need not concern himself about induced winter activity of the bees during cold weather. A certain degree of protection is necessary, during protracted cold periods with temperatures below zero Fahrenheit, for two reasons: First, there is the danger that when the cluster has contracted to its minimum size, thereby increasing its heat retention proficiency to the maximum, the reduced rate of metabolism will not produce sufficient heat to prevent stiffening and subsequent death of a part or all of the bees; second, the hive temperature must be sufficiently high (about $32^\circ$ to $38^\circ$ F.) at intervals in order to permit the movement of the bees onto the reserve supply of honey.

These data have a more practical significance in the wintering of bees in cellars. The temperature within a bee cellar may be controlled within the desired limits during the winter. It is apparent from this study that the desirable temperature in the cellar is the lowest one which will allow for the movement of bees onto new stores when needed. Such a temperature has not been accurately determined in these preliminary experiments.

C. H. Gilbert has found from an experiment being conducted here at the present time that there is a difference between cellar and hive temperature of $7.9^\circ$ F. when the mean cellar temperature for the winter was $38.7^\circ$ F. These figures were obtained in the case of an ordinary 10-frame hive, single story.

The generally recommended cellar temperatures are $42^\circ$ to $46^\circ$ F. Such cellar temperatures, then, would produce hive temperatures of from $50^\circ$ to $54^\circ$ F. At the present time it can only be said that such temperatures within the bee hive are too high by possibly $6^\circ$ to $10^\circ$ F. for the best results.
CONCLUSIONS

From these preliminary experiments it is impossible to draw final conclusions. Certain important tendencies, contrary to previous theory, are displayed. These tendencies are:

1. As the temperature of the air surrounding the bee cluster drops below the temperature of cluster formation, there is a decrease in the rate of metabolism.

2. The activity of cluster formation produces a relatively high rate of metabolism which gradually lowers to a fairly stable constant.

3. Basal metabolism seems to lie in a temperature range between 4° and 8° C.

4. The commonly accepted practice of maintaining cellar temperatures at about 44° F. will not produce a minimum rate of metabolism. Temperatures 6° to 10° F. lower in the cellar will reduce activity and be entirely within the range of safety for the movement of bees onto new stores.

ACKNOWLEDGMENTS

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LITERATURE CITED

(1) Milner, R. D., and Demuth, Geo. S.

(2) Farrar, M. D.

(3) Corkin, C. L.
1930—The Metabolism of the Honeybee Colony During Winter; Bul. No. 175, Wyo. Agricultural Experiment Station.

(4) Rosencrans, C. Z.

(5) Hamilton, W. F.

(6) Betts, Annie D.
1930—The Ingestion of Syrup by the Honey Bee; The Bee World, Vol. XI, No. 8, pg. 85-90.

(7) Merrill, J. H.

(8) Parker, R. L.
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