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# A Study in the Metabolism of the Adult Honey Bee

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# A STUDY IN THE METABOLISM OF THE ADULT HONEY BEE

By

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THE HONEY BEE
(Apis mellifica)

Introduction: The study of metabolism in the higher forms of life has been carried on for many years and much has been found out that has been of great importance in understanding the way things live and grow. It has been only in the last few years that attention has been given to this phase of the work in insects. The extensive work that has been carried on concerning metabolism in man and animals is the basis for the more recent researches on the lower forms including the micro-organisms. Before this work could be carried out on the small forms it was first necessary to develop special technique and instruments which would accurately measure their respiration. The basis of metabolism is the chemical breaking down of food material by the body and the use of the food in carrying on body activity. Most organisms have adjusted their mode of living to the extent that their diet is confined to a rather restricted food range. Such is the case in the animals concerned in this paper.

In the selection of honey bees as subject for metabolism studies, the selection was made in light of their economic importance and not because of the fact that bees would be good material on which to work. Actually, bees, due to their gregarious instincts, are unusually difficult to handle in the manner called for in this type of research. Since this work is some of the first along this line, the results and conclusions can not be taken as at all conclusive but rather as a basis for future study as better technique is developed.

The greater part of this study has been carried on in a micro-respirometer modified after Thunberg 1905 and Trendlenburg 1909. The apparatus was designed for the study of tissue respiration and can be

equally adapted for work with small organisms. The instrument is volumetric in nature and consists of two small respiratory chambers of equal capacity connected together thru stop-cocks by a fine capillary tube. In the capillary is placed a drop of oil and any change in the capacity of either chamber is read on a scale along the length of the capillary tube by observing the movement of the oil drop.

In metabolism studies we are interested in the use of food and the elimination of body wastes. These wastes are carbon dioxide, water and nitrogenous products. In the study of the bees, we need consider only the carbon dioxide and water because the honey bee subsists on a diet almost exclusively of honey. Honey is a mixture of the simple sugars of the formula $C_6H_{12}O_6$. One gram molecule of this sugar requires 6 molecules of oxygen for its combustion and in metabolism gives off 6 molecules of carbon dioxide and 6 parts of water. Since a molecule of one gas is equivalent to a molecule of any other gas, both taken at the same temperature and pressure, in the burning of honey equal volumes of oxygen and of carbon dioxide are involved. In the light of this chemically established fact, a metabolic relationship has been established between the oxygen intake and the carbon dioxide outgo. This relationship is the value for carbon dioxide, divided by the value for oxygen, the resulting ratio being called the respiratory quotient or R. Q. Theoretically this can be calculated for the metabolism of carbohydrates, fats and proteins. The R. Q. of carbohydrates should be (1), protein (0.8), and fats (0.7). These ratios have been borne out in experiments on man and animals and have established the fact that nothing is gained or lost in the body without a compensating force of energy to balance.

The measurement of oxygen consumption was done by absorbing the $CO_2$ in KOH and recording the loss in volume. Carbon dioxide is measured by using sulphuric acid in the chambers and observing the difference between oxygen used and carbon dioxide given off. For good readings with the volumetric apparatus a water bath was constructed which held constant temperature thruout the experiments. The instruments required about one hour to adjust for temperature; therefore, all readings were begun at the end of one hour.

Bees differ from other insects in that they do not hibernate during cold weather but instead form a cluster within their hive. The temperature of this cluster never falls below 14 °C. any time during the year. Sufficient heat is generated within the cluster to maintain this temperature by the consumption of honey and by body activity. In view of this fact several temperatures for experiments were selected that would correspond with known hive temperatures. The following temperatures were used, 0 °C; 14 °C cluster temperature; 21 °C room temperature; 26 °C; 34 °C brood rearing temperature.

Life History: The honey bee belongs to a group of insects that have complete metamorphosis in their development. In this type of development there are four distinct stages in the life of the organism; namely, egg or embryonic stage, larval or feeding stage, pupa or period of transformation, and adult stage. Due to special care during the larval development in the manner and kinds of food supplied, there is a difference in time of development for queen, worker and drone. The number of days required for development being 16, 21 and 24 days respectively, the exact length of time depending somewhat on the hive temperature. Since this

paper deals only with the worker bee, the worker life history will be considered more in detail. The embryonic stage of the worker requires three days. At the end of this time the tiny larva breaks the delicate egg shell and lies free in the bottom of the comb cell. Nurse bees immediately supply it with a quantity of special food obtained from the salivary glands of their own bodies. An excess of food is generally provided and often this floats the small larva. This food is given the first three days of larval life after which a mixture of pollen and honey are added to the diet. The larval growth is very rapid and full growth is attained by the end of six days of feeding. When the larva is fully grown the cell containing the larva is sealed over with a porous capping. The larva then spins a very thin cocoon after which the last larval skin is cast. A period of rest now follows during which time the larval tissues are torn down and replaced by those of the adult bee. This process requires twelve days for completion. At the end of the twenty one days the fully grown adult chews its way thru the capping and emerges as an adult worker. The life of the worker depends on the activity of the individual and lasts from a few weeks in the working season to a number of months in the less active season.

Environment of the hive: Through their gregarious habits, bees have developed a restricted type of home environment especially relative to their temperature requirements. According to Shull, individual bees have a critical temperature of -1.5°C. A bee becomes helpless at 7 to 8°C and only moderately active in temperatures from 8 to 14°C. According to Phillips the lowest minimum temperature maintained in the hive

is about 14°C. As soon as the hive temperature approaches 14° the bees form a ball or cluster, the temperature of which seldom falls below 14° and generally increases as the outside temperature falls. This condition of cluster formation and temperature maintenance means that the bees remain active thruout the year, consuming honey in sufficient quantity to produce heat for the cluster temperature control. Undoubtedly metabolism during the winter is reduced to a minimum by natural heat conservation in the cluster and for the conservation of the food supply.

The second characteristic hive temperature is that temperature maintained in the brood nest during the period of rearing of young bees. Phillips gives 34°C as the normal brood rearing temperature.

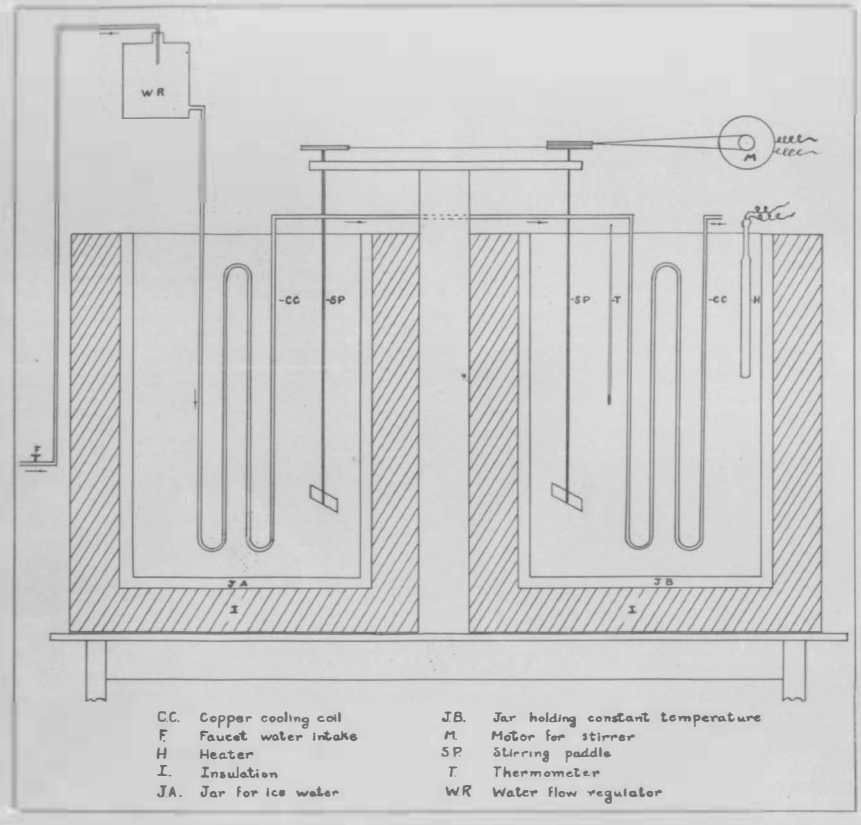
Experimental temperatures: The temperatures known to exist in the hive and several other temperatures were selected as a basis for our metabolism studies. The following Centigrade temperatures have been considered: 0.3; 14.0; 21.0; 26.0; 34.0; 36.0. The temperature of 0.3°C is of course far below what a bee would normally have to cope with. At this temperature bees were motionless with cold and remained so thruout the experiment. As might be expected the rate of metabolism was very low. After about six hours they seemed to become accustomed or adjusted to the extreme cold and very satisfactory readings could be taken over a number of hours time. Most of the readings at this temperature were carried over a period of 24 hours, a few for even a longer time. After the first six hours, readings were quite uniform but they fell slightly towards the end of the period. No experiment was carried on sufficiently long to kill the bees at this

temperature. Bees recovered after exposure for 36 hours.

All of the higher temperatures required the development of special technique relative to the time of exposure and to the number of individuals that could be used in the instruments for each experiment. This change in technique was required because at the higher temperatures the bees were very active, exhausting the oxygen in the chambers very rapidly. This activity also determined the life of the bee in the experiment. At 34° and 36° many of the bees died from exhaustion or starvation before the experiment was complete.

Experimental temperature control: The use of the volumetric microrespirometer required the use of a constant temperature control thruout the experiments. This condition was provided for by the use of a water bath having a temperature control. The instruments were placed below the surface of the water in the bath with only the ends of the side arms extending above the surface of the water. This arrangement placed the working parts of the microrespirometer under constant temperature control, yet left the interior of the apparatus exposed to atmospheric pressure as long as the stop-cocks were opened to the outside.

The apparatus for temperature control used in these experiments is figured in the diagram on figure 1, page 7. Two twenty-gallon jars were placed side by side and each was provided with about four inches of insulating material on the sides and bottom. Each jar was fitted with a copper cooling coil made from fifteen feet of one-fourth inch tubing. The two coils were connected by means of rubber tubing. The water was circulated thru the coils by carrying a stream of water from a faucet up into an over-head jar. The water flowed by gravity from




C.C. Copper cooling coil
F. Faucet water intake
H Heater
I. Insulation
JA. Jar for ice water
JB. Jar holding constant temperature
M. Motor for stirrer
SP Stirring paddle
T Thermometer
WR Water flow regulator

Figure I

this jar through the coils and out through the over flow into the sink. The faucet could be so regulated that the water circulated through the coils at a uniform rate. The first jar through which it circulated was maintained as an ice water bath. Ice was added from time to time to off set that melted by the cooling coil. Water passing in the coils of this jar become ice cold and entering the second jar in this condition at a uniform rate, served to keep the water bath of the second jar at any desired constant temperature, below that of room temperature. For temperatures higher than those normal in the laboratory a heating unit was added to the second jar. Excess heat from this unit was carried off by the water in the cooling coils. A stirring paddle in each jar, driven by a small electric motor, kept the water in constant motion at all times. The above arrangement gave satisfactory temperature control for the temperature range used in these experiments.

Digestion in the honey bee: According to Phillips (1924) the power of digestion in bees lies within a very restricted range. In a series of experiments he fed chemically pure foods and found that bees could digest certain foods while other foods caused apparent starvation. He attributed this fact to the lack of certain enzymes for the hydrolysis of those foods on which the bees had starved. Of the simple sugars he found the bees could utilize dextrose and levulose, the two common components of honey. They were unable to utilize mannose or galactose.

Of the pentose sugars they could not use such as xylose and arabinose.

Of the sugars composed of two simple hexose sugars, they are able to use cane sugar and maltose. They were unable to live on lactose or

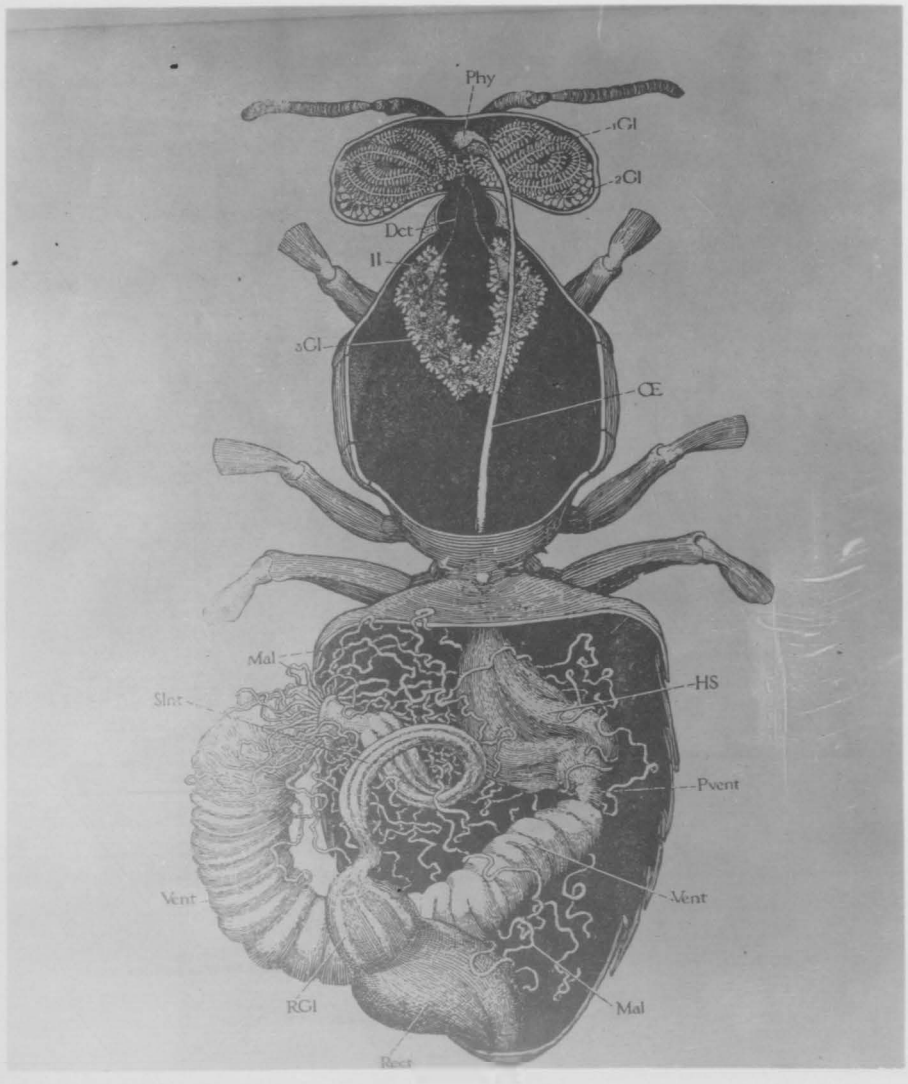

Phy
1Gl
2Gl
Dct
II
3Gl
Œ
Mal
Slnt
HS
Pvent
Vent
Vent
RGl
Mal
Rect

Figure 2

milk sugar, dying as fast as though starved. A rare sugar, trehalose, found in fungi and certain mannas of the old world, was as readily digested as cane sugar.

Of the trisaccorides the bees thrived on melezitose and starved on raffinose. Melezitose is a rare sugar which has been found recently in certain honey dews. All of it probably is derived from coniferous trees.

Among other carbohydrates tested the following were found not available to the bees: dextrins, several starches, soluble starch, inulin, glycogen and other complex carbohydrates.

For the digestion of carbohydrates there are perhaps two enzymes which make them available, namely, invertase and maltase.

Proteins available as food for bees come almost entirely from pollen grains. These grains must first be crushed by the mandibles of the worker or nurse bees before the contents are available to either the adult or young bees. According to Peterson (1913) and Snodgrass (1925) the ventriculus contains the enzyme tyrosine. Pavlowsy and Zarin (1923) found pepsin, trypsin and chymosin in the ventriculus, all being well known enzymes of proteids.

Although fat is present in the tissue of both developing and adult bees, as far as is known, fat is not utilized by the bees in the digestive process. In the case of the developing bees much of the fat is used up during metamorphosis. Fat is apparently not absorbed in the alimentary canal.

Alimentary canal of the adult bee: The anatomical structures of the honey bee that have to do with assimilation of food and the elimination of body wastes are figured on page 9, figure 2. The food is

taken into the body by means of the sucking mouth parts which extend in front of the pharynx. The pharynx aids the mouth parts with a sucking action that causes the food to ascend into the pharynx. The pharynx joins directly to a narrow tube called the esophagus which carries the food posteriorly through the thorax and into the expanded end of the esophagus or honey stomach. This structure has no digestive glands in its walls, is lined with a simple epithelium, and is supported by bands of transverse and longitudinal muscle fibers. Under normal conditions a bee carries some honey in this stomach. This honey serves as a reserve supply of food whenever needed by the body. The food carried in this structure is believed by the writer to be directly responsible for one variation of body weights of individuals. In some metabolic experiments, where activity was inevitable, this factor of reserve food accounted for the varying length of life found among individuals under experimentation. Those with a full honey stomach lived thruout and after an experiment, while those without either became inactive during the experiment or died before the experiments were terminated.

In the posterior part of the honey stomach is a valve known as the proventricular valve. This valve prevents food from returning into the honey stomach once it has entered the ventriculus or true stomach. The ventriculus is a U-shaped tube having rather convoluted muscular walls. It is lined with digestive and secretory tissues. Probably the greatest part of the digestion and absorption takes place in this structure.

Just posterior to the ventriculus is the small intestine. Surrounding the anterior end of this tube are the individual openings of the malpighian tubules. These are long slender tubes that lie

wrapped and coiled together within the body cavity. Their function is that of collecting the body wastes and excreting them into the alimentary canal. The posterior end of the small intestine is enlarged to form the sac-like rectum. Some absorption may take place in the rectum, but its principal function in the case of the honey bee is to store feces at times for several months. Since the bee voids its feces only when in flight, it is probable that the rectum contains some feces at all times, except immediately after flight. Bees taken from the winter cluster for experimentation undoubtedly contained considerable feces, a fact which would account for a second variation in weight for bees used at this time of year. The weight depended on the relative activity of the cluster and the time intervening since the last flight.

Honey as a food: The greater part of the food used by the honey bee consists of honey. Since the metabolic rate is dependent on this source of energy, a brief review of the chemical composition of honey is included in this paper.

Honey is 81.2 percent carbohydrate, 0.4 percent protein, 0.0 percent fat, 17.79 percent water, and the remaining .61 percent is mineral and undetermined matter. According to Phillips, a bee can use all the carbohydrate content of honey except dextrin. The protein content of honey alone is certainly not sufficient to supply the needs for tissue replacement. Undoubtedly additional protein is secured from pollen grains which are rich in protein. As yet it is not known what the protein requirement of the bee may amount to. Fat seems to play no role in the honey bee's digestion.

Chemical analysis. Browne gives the chemical analysis of honey as follows:

| | | |
|---|---|---|
| Water | 17.79 | percent |
| Sucrose | 1.90 | " |
| Levulose | 40.50 | " |
| Dextrose | 34.43 | " |
| Dextrin | 1.51 | " |
| Ash | 0.18 | " |
| Undetermined | 3.73 | " |

The undetermined matter is made up of iron, lime, sodium, sulphur, magnesia, potassium, manganese, phosphoric acid, pollen grain albumen, aromatic bodies, and various other bodies of indefinite and unknown character.

Sherman gives the ash constituents as follows:

| | | |
|---|---|---|
| Calcium | 0.004 | percent |
| Magnesium | 0.018 | " |
| Potassium | 0.386 | " |
| Sodium | 0.001 | " |
| Phosphorus | 0.019 | " |
| Chlorine | 0.029 | " |
| Sulphur | 0.001 | " |
| Iron | 0.007 | " |

Rose gives the caloric value of honey as 3.26 calories per gram.

Bees feeding on honey alone have a diet of 98.39 percent carbohydrate and water. The water does not enter into the digestive process chemically, so the metabolism of sugars only will be considered. According to well known chemical formulae, the following is the chemical

oxidation of a typical sugar.

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6\ H_2O$$

Calculations of molecular weights are as follows:

| Sugar | Oxygen | Carbon-dioxide | Water |
|---|---|---|---|
| Carbon 6 x 12 = 72 | ---------- | 6 x 12 = 72 | ---------- |
| Hydrogen 12 x 1 = 12 | ---------- | ---------- | 12 x 1 = 12 |
| Oxygen 6 x 16 = $\frac{96}{180}$ | 12 x 16 = $\frac{192}{192}$ | 12 x 16 = $\frac{192}{264}$ | 6 x 16 = $\frac{96}{108}$ |

Since one gram of honey furnishes 3.26 calories, 180 grams will furnish 586.8 calories of heat. The factor for conversion of oxygen into liters is 0.6997 and for carbon-dioxide 0.5089.

192 molecules of oxygen x 0.6997 = 134.34 liters.

264 molecules of carbon-dioxide x 0.5089 = 134.34 liters.

According to the previous equation, the metabolism of 180 grams of sugar produced 586.6 calories and in so doing used 134.34 liters of oxygen, and gave off 134.34 liters of carbon-dioxide. Each liter of oxygen then produced 4.368 calories of heat.

The metabolic ratio known as the respiratory quotient is based on the above factors. In this ratio the amount of carbon-dioxide eliminated is divided by the amount of oxygen consumed. In the above reaction 6 molecules of each gas entered into the reaction and since a molecule of one gas equals a molecule of any other gas under constant temperature and pressure, this ratio is established.

$$\frac{CO_2}{O_2} \quad \text{or} \quad \frac{134.34 \text{ liters}}{134.34 \text{ liters}} = 1.00$$

In the case of carbohydrates, the respiratory quotient would theoretically be one (1), if the chemical process went to completion.

A similar analysis relative to the chemical breaking up can be applied to the fats and proteins, in which the respiratory quotients work out theoretically to be less than one (1).

Description of the instruments: The instrument used almost exclusively in these experiments is known as the Thunberg microrespirometer. Several modifications to Thunberg's original design have been made. The capillary tube of his instrument, instead of being straight, was bent slightly down in the center. Recent experiments have shown the straight capillary tube to be equally satisfactory. Trendelenberg added a side arm to each respiratory chamber in order that the gases might be analysed or exchanged without altering the instrument during an experiment. Fundamentally, however, the apparatus is little different from the original design. The first use of the apparatus was for measuring the minute gas exchange between Algae and a small Sea-Anemone. Later workers have adapted its use to other fields requiring micro measurements of gases; Trendelenberg for small invertebrates, Krog and Van der Heyde for insects, Hopkins for tadpoles and muscle-tissue, and Fenn for nerve-tissue. This type of apparatus adapts itself readily to the work with bees, particularly to the immature stages such as egg, larva and pupa. Since the development of individuals is very rapid, hourly changes are very important, and such may be obtained by making hourly readings over a period of the development.

In my experiments I used several different instruments, each having a different capacity of the capillary tube and of the respiratory chambers. I found this change of instruments necessary because of the greatly increased rate of respiration of the bees at the higher temper-

atures. I also found it necessary to reduce the number of individuals used in the respective instruments as the temperature was raised from low to high. For example, an instrument chamber with capacity for ten bees at zero degrees could handle but two bees at 34 degrees Centigrade.

My instruments were of two types with reference to the method of connection of the respiratory chambers with the instrument. The smaller instrument, figure 3, page 17, was made with two 21.48 cc. capacity chambers, of as nearly equal capacity as possible. These two chambers were connected to the ends of the capillary tube by a ground glass union. Each time the instrument was used a paraffin-beeswax seal was heated into the ground glass union, which made a permanent seal for the length of the experiment. Little trouble was experienced with the breaking of this seal from the expension and contractions caused by temperature changes. The two equal chambers were connected thru a three-way stop-cock by a capillary tube having a capacity of 1.324 cu. mm. per mm. of length. Within this capillary tube an alcohol bubble of about one cm. length was maintained. Back of the capillary tube was fitted a millimeter scale against which any movement of the alcohol drop could be noted. Short side arms extended upward from the three-way stop-cocks. To prevent the organisms from falling into the solutions used in the chambers, two glass supports were made which were of as nearly equal weight as possible. One of these supports was used in each chamber whenever the experiments were in progress. The entire instrument was supported in such a way that the tips of these side arms extended above the surface of the water in the water bath. While the instruments were acquiring constant temperature within this

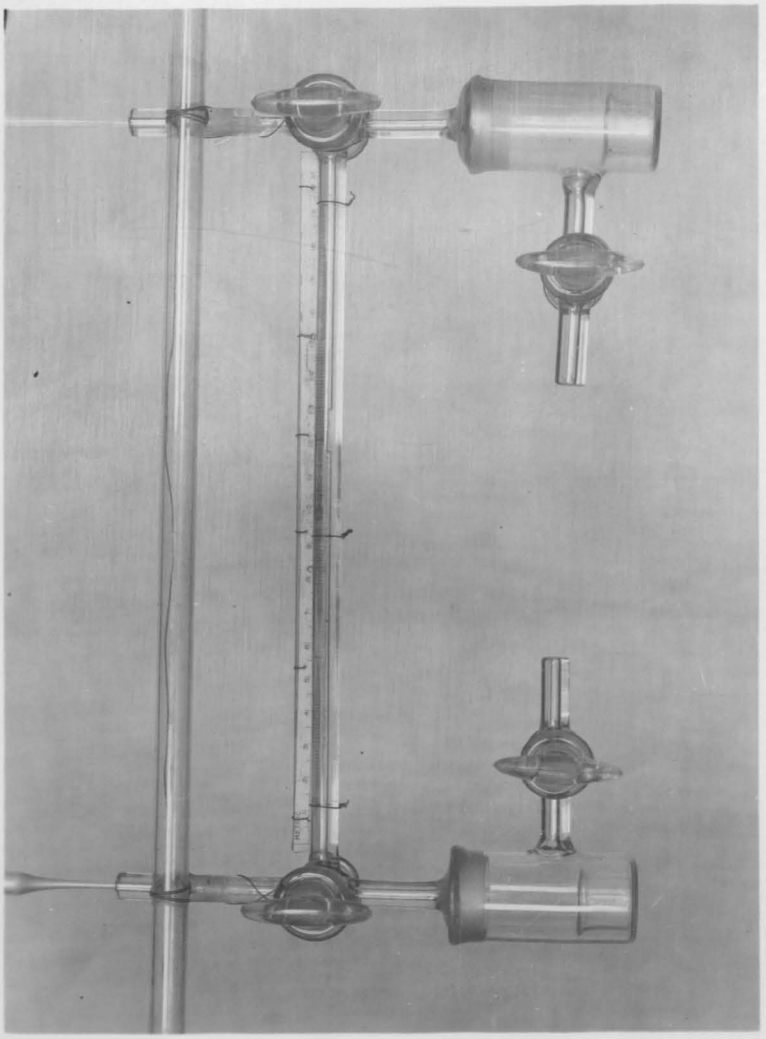


Figure 3

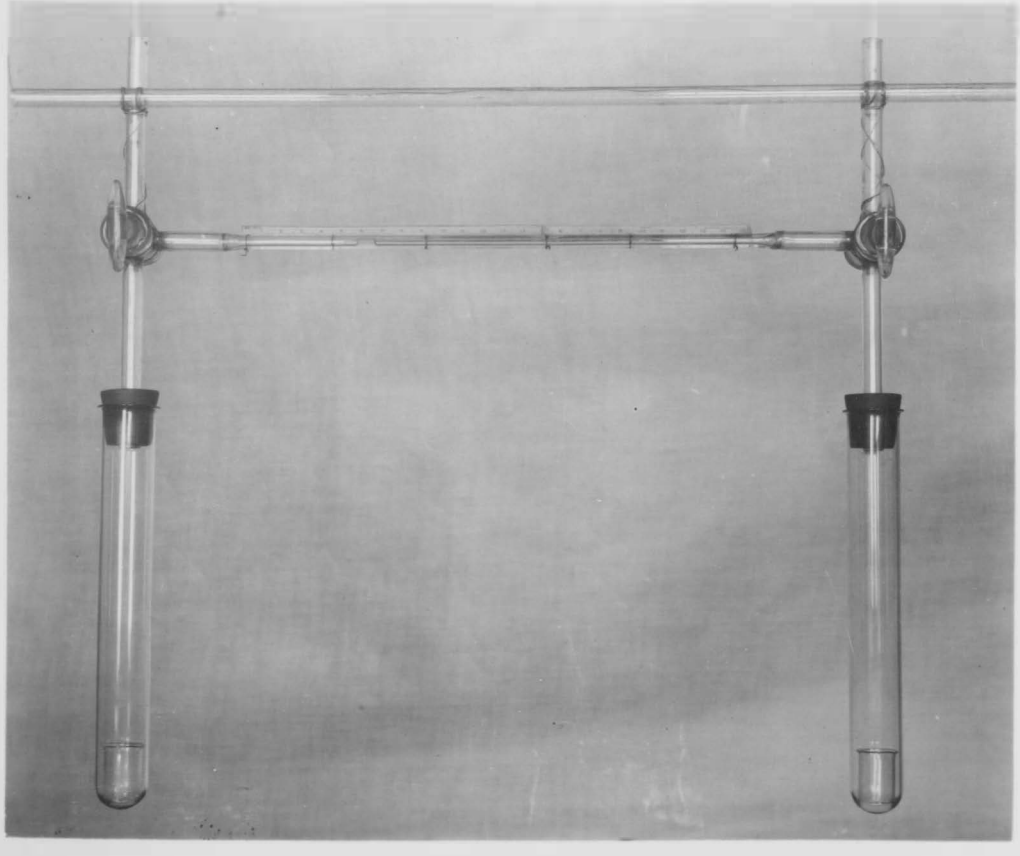


Figure 4

water bath, the stop-cocks were turned so that the interior of the respiratory chambers were exposed to atmospheric pressure. A period of one hour was required to establish equilibrium between the instruments and the water bath.

The second instrument differed from the one described above in capacity and in chamber connections. The respiratory chambers were large test tubes of 80 cc. capacity and connected with the capillary tube by means of rubber stoppers. The rubber stoppers made satisfactory seals in most cases, but required careful technique in adjustment to avoid slipping caused by the temperature changes. This instrument, figure 4, page 18, had a capillary tube of a capacity of 4.69 cu. mm. per mm. of length.

A third instrument used to some extent, was very similar to the one just described except that the capillary capacity was 9.33 cu. mm. per mm. of length. For these last two instruments with the larger capacity, a high grade petroleum oil was used as an indicator bubble instead of an alcohol bubble as in the smaller instrument. The essential features of the oil used in the capillary tube as an indicator bubble was that it be free flowing with a minimum resistance, at the various temperatures used.

Measurement of Oxygen: The following technique was used in the measurement of oxygen consumption: An equal amount of 40 percent potassium hydroxide was placed in the bottom of each respiratory chamber. The glass support for the organisms was then lowered into each chamber. The desired number of bees were confined in one chamber and the other chamber was left empty. Both chambers were attached to

the instrument proper and the connections made absolutely tight. From experiment to experiment the opposite chambers were used for the bees in order that any possible error of capacity might be off-set. The entire instrument (except for the tips of the side arms) was then submerged in the water bath for one hour before closing the chambers to atmospheric pressure. After the hour had elapsed the stop-cocks were turned to connect the chambers with the capillary tube and the movement of the oil drop recorded. In this case any carbon-dioxide given off was absorbed in the potassium hydroxide. The slight reduction in pressure within the chambers, altho computable, was considered of no importance for experiments of short duration. Since the pressure adjustment is equal between the chamber containing the organisms and the opposite empty chamber, it is obvious that the oil bubble will move just one-half as fast as it would normally move with one chamber exposed to the atmosphere. Consequently, all readings in the final analysis are multiplied by two to give the true movement of the bubble.

Measurement of Carbon-dioxide: In Thunberg's experiment, the carbon-dioxide elimination is measured in an indirect method. In these experiments, dilute sulphuric acid was used in the respiratory chambers instead of potassium hydroxide as used for the absorption of carbon-dioxide. The sulphuric acid absorbs neither oxygen nor carbon-dioxide. Since the gases were at equal pressure, the difference between the oxygen consumption and the carbon-dioxide elimination was read by the movement of the oil bubble. In case the oxygen consumed was greater than the carbon-dioxide given off, the movement was toward the organism chamber, or if the oxygen consumption was less, the movement was toward the blank chamber. Knowing the difference between the two gases, and the amount of oxygen consumption from

previous experiments, the carbon-dioxide elimination was determined.

Standardization of metabolic results: All tables and calculations of the metabolic rate in these experiments have been reduced to the standard temperature and pressure of 0 degrees centigrade and 760 mm. mercury. The reductions of all conditions to standard conditions is obviously necessary in order that the various experimental results may be compared on an equal basis.

Several well known laws of physics have been involved in order to make these reductions. They are reviewed briefly below:

Boyle's Law: The density of a gas is directly proportional to its pressure at a constant temperature. The formula used for this correction is the observed pressure in mm. mercury, divided by 760, where the temperature remains constant.

Charles or Gay-Lussac's Law: The volume of a gas is very nearly proportional to its absolute temperature when the pressure is kept constant. When the volume is kept constant, the pressure of a gas is proportional to the absolute temperature. It is therefore necessary to correct for temperature since the volume of a gas is always expressed at a standard temperature of 0 degrees Centigrade. The formula for this correction is $\frac{1}{1 + 0.00367\ t}$, t being the observed temperature in degrees Centigrade and 0.00367 the reciprocal of -273 degrees, which is absolute zero.

Avagadro's Law: The number of molecules per cubic centimeter is the same in all gases at the same temperature and pressure. This law is the basis for all calculation of the metabolic ratio known as the respiratory quotient.

The complete formula for correction of the results obtained from

the Thunberg apparatus is $\frac{OP}{760} \quad \frac{1}{(1 + 0.00367\ Z)} \quad \frac{2\ V\ R}{X\ T}$ = cubic millimeters per hour per individual at 0 degrees Centigrade and 760 mm. mercury. OP is the Observed Pressure in mm.; Z is the Observed Temperature in zero degrees Centigrade; V is the Volume of capillary per mm. length; R is the Observed Movement of the oil droplet; X is the Number of Individuals used; T is the Length of Time used in experiment in hours.

As an example, a typical experiment is shown in details:

OP = 719.0 millimeters mercury

Z = 21.0 degrees Centigrade

V = 9.33 cubic millimeters

R = 97.0 millimeters on scale

X = 6 bees

T = 1 hour

According to formula: $\frac{719 \times \qquad 1 \times \qquad 2 \times 9.33 \times 97}{760 \times (1 \quad 0.00367 \times 21) \times 6 \times 1} =$

267.5 cubic millimeters.

Weight of bees relative to metabolism: Most workers in the field of metabolism base their metabolic coefficients on the body weight of the animals studied. In the case of bees it was found useless to attempt to weigh the individuals used in the experiments due to a wide variation of individuals from their true body weight caused by the load that they were carrying. A few bees were weighed at the beginning of the experiment and such a wide variation was found that it was decided to rely on the figures determined as minimum flying weight for workers, as a basis for metabolic calculation. Park (1924) brought up to date previous works on the weights of bees and by a series of his own de-

terminations found what appeared to be the minimum flying weight of worker bees. He concludes that 82 mgs is the approximate minimum flying weight of an Italian worker bee. This figure of 82 mgs is the figure used in all calculation in this paper where the weight of the individual or of groups of bees is involved.

Source of bees used in the experiments: A moderately strong colony of bees was permanently established in the attic of the laboratory where their hive conditions were similar to those of a well-packed colony wintering out of doors. The colony reared brood until December, and then completely ceased brood-rearing until the middle of March. Brood-rearing was carried on vigorously during the latter part of March but this was followed by a broodless period the latter part of April and the first part of May. The broodless period may be explained by the lack of pollen in the hive at this time, for brood-rearing began soon after pollen became available out of doors. Thruout the year the bees needed in the experiments were taken from this hive but were not returned after being used in an experiment.

Since the experiments were carried on over many weeks and under a variety of activity conditions within the hive, the results obtained under various experimental conditions might be misleading unless they are carefully analyzed and restricted. Therefore, results are conclusive only for that period of the yearly cycle from which the bees were taken for the experiments. Only by carrying on experiments for all temperatures thruout the yearly cycle can sufficient data be obtained for a complete understanding of honey bee metabolism. Judging from the variations in experimental results, the writer believes that the metabolic rate is far from constant thruout the yearly cycle.

Low temperatures: The aim of the experiments with low temperatures was to place the bees under such conditions that their metabolism would become basal or below basal. At 14° Centigrade or higher the factor of activity is involved bringing in a great source of error in results. 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. At the temperature of 0.3° Centigrade there was no noticeable activity of the bees after the first three minutes of exposure. The bees had been taken from the winter cluster that had a temperature of not less than 14° Centigrade and at 0.3° Centigrade their metabolism decreased hourly to that shown in figure 5, page 26, and figure 6, page 27. During the first six hours the metabolism decreased rapidly, but about the seventh hour a very gradual decline was established. The respiratory quotient also decreased with the time of exposure, and after five hours this ratio dropped below that of average carbohydrate metabolism and approached a protein ratio of metabolism. This fact suggests that honey was no longer being metabolised and that the bees were existing on their body tissues. The experiments at low temperatures were carried on over a twenty-four hour period, but the last twelve hours of readings are averages and not hourly readings. These averages are plotted on Figure 5 as a dotted line, but it is reasonable to believe that metabolism falls within the range shown by the dotted lines.

Figure 7, page 28, gives the average results for the twenty-four hours of exposure to a low temperature. The average oxygen consumption for twenty-nine bees over a total of 194 hours exposure was 6.89 cubic millimeters per hour. The carbon-dioxide output was 5.38. The

average respiratory quotient was 0.783, closely approaching the respiratory quotient of protein metabolism. The bees dissected after twenty-four hours of exposure still had a quantity of honey in their honey stomachs. If digestion did continue at this temperature it evidently did so very slowly.

Winter cluster temperature: The aim of the experiments at 14° Centigrade was to secure data as to what the metabolism might be in the winter cluster. In the cluster, bees always appear quiet and metabolism is evidently greatly reduced. Experimentally bees did not remain quiet at this temperature and consumed almost as much oxygen as they did at much higher temperatures. This may possibly be explained by the fact that the bees were not in their normal surroundings. The constant exposure to 14° Centigrade with the constant removal of all heat generated by their activity may have made them restless. The rapid increase in oxygen consumption from 29.64 cubic millimeters at 0.3° Centigrade to 265.2 cubic millimeters at 14° Centigrade is rather striking. At just what temperature the rapid increase takes place has not been determined but it is certainly not a gradual rate of increase as the temperature increases. The complete data for results of the experiments at 14° Centigrade is shown on Figure 8, page 29, and Figure 9, page 30. These tables are the results of working with two different instruments. Altho there appears a difference in oxygen consumption and carbon-dioxide output, the respiratory quotients are both about 0.94±, or within the range of carbohydrate metabolism.

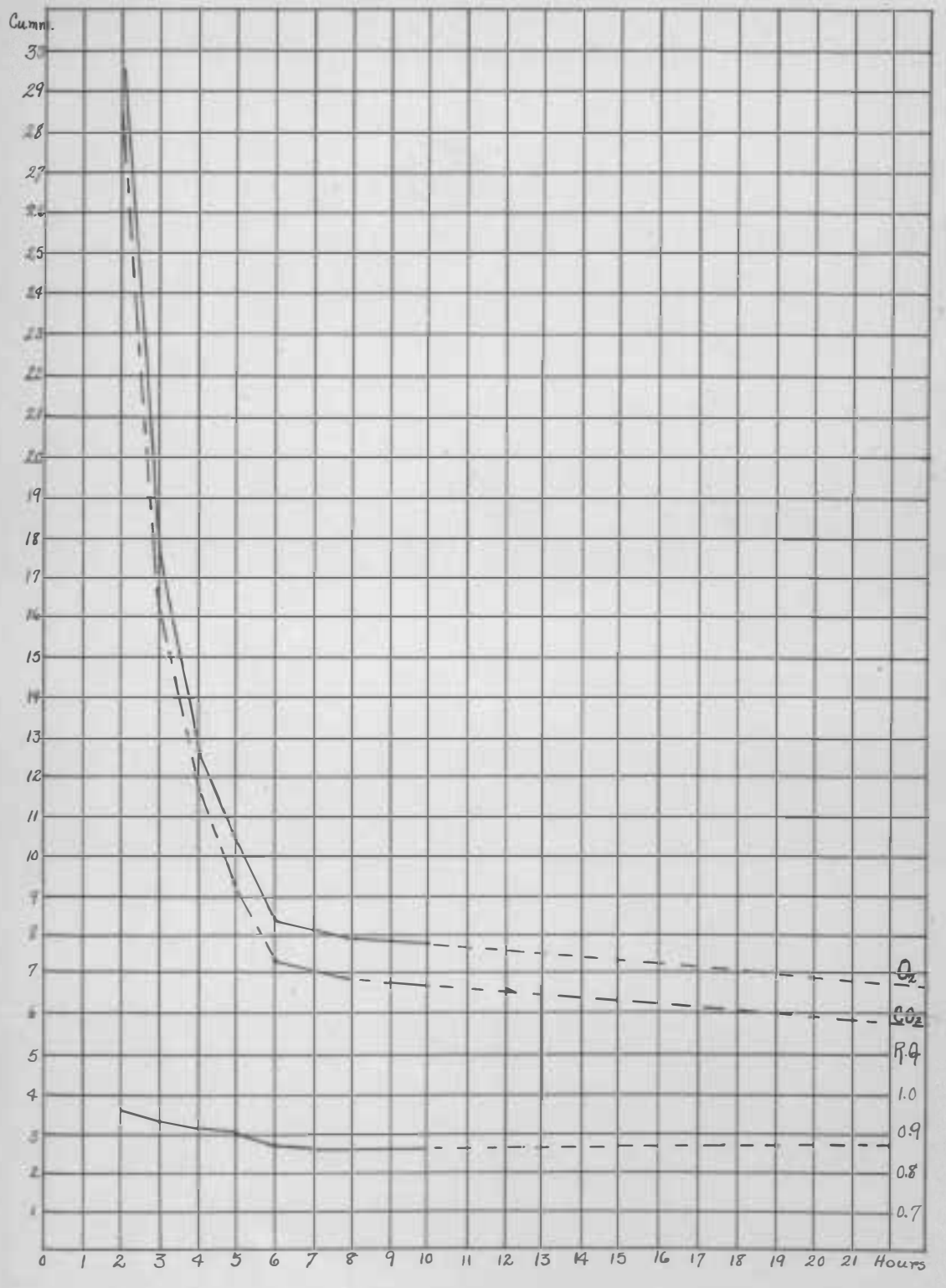



FIGURE 5 REACTION OF BEES TO TEMPERATURE OF 0.3°C.

Table giving the average results of gas exchanges for eleven oxygen and four carbon dioxide experiments at 0.3° Centigrade - 760 mm..

| Hour of Reading | Respiratory Quotient | Oxygen | Carbon Dioxide |
|---|---|---|---|
| 2 | 0.962 | 29.64 | 28.56 |
| 3 | 0.939 | 17.49 | 16.41 |
| 4 | 0.915 | 12.76 | 11.68 |
| 5 | 0.905 | 10.35 | 9.27 |
| 6 | 0.870 | 8.29 | 7.21 |
| 7 | 0.868 | 8.20 | 7.12 |
| 8 | 0.863 | 7.91 | 6.83 |
| 9 | 0.863 | 7.90 | 6.82 |
| 10 | 0.853 | 7.74 | 6.66 |

Figure 6.

Oxygen consumption and carbon-dioxide output in cubic millimeters per hour per bee at 0.3° C. 760 mm. over a period of long exposure.

Oxygen = 6.89. Carbon-dioxide = 5.38. Respiratory Quotient = 0.783.

| OXYGEN | | | |
|---|---|---|---|
| Date | No. Bees | Hours Exposure | Average cu. mm. per hour |
| January 4, 1927 | 3 | 23 | 8.57 |
| " 5, " | 3 | 20 | 6.21 |
| " 6, " | 3 | 21 | 7.78 |
| " 12, " | 3 | 23 | 7.66 |
| " 14, " | 3 | 20 | 5.83 |
| " 17, " | 4 | 22 | 6.87 |
| " 18, " | 4 | 21 | 7.27 |
| " 19, " | 3 | 21 | 5.42 |
| February 4, " | 3 | 23 | 6.44 |
| TOTAL | 29 | 194 | AVERAGE 6.89 |
| OXYGEN minus CARBON-DIOXIDE | | | |
| January 22, 1927 | 3 | 21 | 1.58 |
| " 24, " | 3 | 21 | 1.93 |
| " 25, " | 4 | 20 | 1.98 |
| " 26, " | 3 | 19 | 1.90 |
| " 27, " | 4 | 20 | 2.44 |
| " 28, " | 3 | 22 | 0.97 |
| " 31, " | 3 | 22 | 0.83 |
| February 1, " | 3 | 21 | 1.27 |
| " 2, " | 3 | 22 | 1.24 |
| " 3, " | 3 | 20 | 0.96 |
| TOTAL | 32 | 208 | AVERAGE 1.51 |

Figure 7.

Oxygen consumption and carbon-dioxide output in cubic millimeters per bee at zero degrees Centigrade - 760 mm.

Oxygen = 265.20. Carbon-dioxide = 248.46. Respiratory Quotient= 0.937.

| Date | No. Bees | Temperature | Cu. mm. per hr. |
|---|---|---|---|
| **OXYGEN** | | | |
| February 21, 1927 | 6 | 14.0 | 247.5 |
| " 22, " | 6 | 14.0 | 253.5 |
| " 22, " | 6 | 14.5 | 306.0 |
| March 14, " | 6 | 14.3 | 199.5 |
| " 14, " | 6 | 14.3 | 148.2 |
| " 14, " | 6 | 14.3 | 573.0 |
| " 15, " | 6 | 14.0 | 300.0 |
| " 15, " | 6 | 14.5 | 93.8 |
| AVERAGE | | 14.23 | 265.2 |
| **OXYGEN minus CARBON-DIOXIDE** | | | |
| February 16, 1927 | 5 | 14.0 | 20.55 |
| " 17, " | 6 | 14.1 | 16.93 |
| " 18, " | 6 | 13.4 | 16.83 |
| March 1, " | 6 | 14.0 | 14.20 |
| " 10, " | 6 | 14.0 | 25.00 |
| " 12, " | 6 | 14.1 | 6.92 |
| AVERAGE | | 14.1 | 16.74 |

Figure 8.

Oxygen consumption and carbon-dioxide output in cubic millimeters per bee per hour at zero degrees Centigrade - 760 mm.

Oxygen = 322.3. Carbon-dioxide = 314.6. Respiratory Quotient = 0.945.

OXYGEN

| Date | No. Bees | Temperature | Cu. mm. per hr. |
|---|---|---|---|
| February 16, 1927 | 6 | 14.0 | 174.3 |
| " 17, " | 6 | 14.1 | 269.1 |
| " 18, " | 6 | 13.4 | 257.5 |
| March 14, " | 6 | 14.3 | 615.0 |
| " 14, " | 6 | 14.3 | 472.0 |
| " 14, " | 6 | 14.3 | 244.0 |
| " 15, " | 6 | 14.0 | 414.5 |
| " 15, " | 6 | 14.0 | 132.6 |
| AVERAGE | | 14.2 | 322.3 |

OXYGEN minus CARBON-DIOXIDE

| | | | |
|---|---|---|---|
| February 21, 1927 | 6 | 14.0 | 20.0 |
| " 22, " | 6 | 14.5 | 19.5 |
| " 22, " | 6 | 14.0 | 22.8 |
| March 10, " | 6 | 14.0 | 6.9 |
| " 12, " | 6 | 14.1 | 19.5 |
| AVERAGE | | 14.1 | 17.7 |

Figure 9.

Room temperatures: The metabolism at 21° Centigrade was found to be similar to but slightly higher than that of 14° Centigrade. The complete data for the experiments at this temperature is shown on figure 10, page 33, and figure 11, page 34. Altho the oxygen consumption and carbon-dioxide output differ but little from that of 14° Centigrade, the respiratory quotient is decidedly lower. As two different instruments were used under similar conditions, and a similar decrease was recorded, it eliminates the likelihood of experimental error and suggests a possible physiological condition present at that time of year different from that of all other experiments. To check this point additional experiments were carried out at 21° Centigrade on August 3 and 4 to see if the low respiratory quotient could be duplicated. Experiments were completed on drones with a resulting respiratory quotient of 0.93 . Eight experiments were completed on field going workers with a resulting respiratory quotient of 0.915. Thus both sets of experiments gave results much higher than those of April. Two facts were brought out by the results of the check experiments; first, that the rate of oxygen consumption during the April experiments was much lower than that obtained in the August check experiments; and second, that the resulting difference of respiratory quotients obtained must be explainable thru a physiological difference in the bees at the time of the experiments.

The condition of the colony from which the bees were taken in April may offer a possible solution to above result. At this time the colony had its smallest number of bees, because of the removal of bees for experiment purposes thruout the winter. At this time also the colony was rearing brood as rapidly as it could be cared for by the workers present

and it is entirely possible that most of the adult bees were helping with the rearing of the brood. As was mentioned previously, the food supplied to honey bee larvae is derived mainly from the salivary glands of the nurse bees. Food must therefore be previously digested by the nurse bees and excreted thru their salivary glands before it is given to the larvae. According to Phillips, the composition of this food as given to the worker larvae is as follows:

| | Proteins | Fat | Sugar |
|---|---|---|---|
| Workers under 4 days | 53.38 | 8.38 | 18.09 |
| Workers over 4 days | 27.87 | 3.69 | 44.93 |

It is a well known fact that pollen is necessary for brood rearing. The protein content of honey is far too low to furnish the quantity of protein matter necessary, so the pollen must be the source of the proteins found so abundantly in the larvae food. Nurse bees must digest the pollen before it can be available to the larvae and the metabolism of the pollen proteins would cause a decrease in the respiratory quotient in the case of the nurse bees. It is entirely possible that nurse bees were among those used in the April experiments and that they may have been responsible for the resulting respiratory quotient at that time.

It is apparent from the experiments carried on in August when field going workers and drones were used that at 21.0° Centigrade their respiratory quotients were normal in respect to the other temperatures for carbohydrate metabolism. Temperature alone was certainly not responsible for the decreased respiratory quotient during April. The above condition suggests a problem in honey bee metabolism relative to the age of the worker bee. Such conditions were not considered in this paper.

Oxygen consumption and carbon dioxide output in cubic millimeters per hour per bee at zero degrees Centigrade - 760 mm.

Oxygen = 308.0. Carbon-dioxide = 275.9. Respiratory quotient = 0.892.

| Date | No. Bees | Temperature | Cu. mm. per hour |
|---|---|---|---|
| OXYGEN | | | |
| March 24, 1927 | 6 | 21.5 | 282.5 |
| " 25, " | 6 | 23.4 | 327.0 |
| " 25, " | 6 | 23.5 | 287.0 |
| " 29, " | 6 | 21.0 | 267.5 |
| " 30, " | 6 | 21.0 | 398.5 |
| April 5, " | 6 | 21.0 | 261.0 |
| " 6, " | 5 | 21.0 | 332.5 |
| AVERAGE | | 21.2 | 308.0 |
| OXYGEN minus CARBON-DIOXIDE | | | |
| April 7, 1927 | 5 | 21.5 | 21.6 |
| " 7, " | 5 | 21.2 | 43.8 |
| " 9, " | 5 | 21.0 | 35.2 |
| " 11, " | 5 | 21.0 | 26.0 |
| " 12, " | 5 | 21.0 | 39.9 |
| " 13, " | 6 | 21.0 | 30.9 |
| " 13, " | 6 | 21.5 | 32.3 |
| " 14, " | 6 | 21.0 | 34.6 |
| " 15, " | 6 | 21.5 | 29.6 |
| " 15, " | 6 | 21.0 | 35.0 |
| " 16, " | 6 | 20.5 | 24.4 |
| AVERAGE | | 20.1 | 32.1 |

Figure 10.

Oxygen consumption and carbon-dioxide output in cubic millimeters per bee per hour at zero degrees Centigrade - 760 mm.

Oxygen = 334.0. Carbon-dioxide = 291.7. Respiratory Quotient = 0.874.

| OXYGEN | | | |
|---|---|---|---|
| Date | No. Bees | Temperature | Cu. mm. per hour |
| March 24, 1927 | 6 | 21.5 | 348.0 |
| " 25, " | 6 | 23.4 | 260.0 |
| " 25, " | 6 | 23.5 | 274.0 |
| " 29, " | 6 | 21.0 | 409.0 |
| " 30, " | 6 | 21.0 | 437.0 |
| April 5, " | 6 | 21.0 | 274.0 |
| " 6, " | 5 | 21.0 | 427.0 |
| " 6, " | 5 | 20.9 | 343.0 |
| AVERAGE | | | 334.0 |
| OXYGEN minus CARBON-DIOXIDE | | | |
| April 7, 1927 | 5 | 21.5 | 60.0 |
| " 7, " | 5 | 21.2 | 19.2 |
| " 9, " | 5 | 21.0 | 56.0 |
| " 11, " | 5 | 21.0 | 46.8 |
| " 12, " | 5 | 21.0 | 45.2 |
| " 13, " | 6 | 21.0 | 43.2 |
| " 13, " | 6 | 21.5 | 39.1 |
| " 14, " | 6 | 21.0 | 52.5 |
| " 15, " | 6 | 21.5 | 32.7 |
| " 15, " | 6 | 21.0 | 36.3 |
| " 16, " | 6 | 20.5 | 34.4 |
| AVERAGE | | 21.1 | 42.3 |

Figure 11.

Twenty-six degrees Centigrade: Two methods for gas measurement were used at this temperature. The results for the Thunberg apparatus are given in figure 12, page 36. In these experiments the oxygen consumption was slightly lower than that found for 21° Centigrade. The resulting oxygen factor was 299.9 cubic millimeters per bee per hour and the carbon-dioxide factor was 283.8 cubic millimeters. This gave a respiratory quotient of 0.94 or well within that of carbohydrate metabolism.

The second method used was the one known as the "Haldane and Pembrey Gravimetric Method". This method involved the measurement by weight of the carbon-dioxide and water given off from the respiratory chamber by absorbing them in soda lime and sulphuric acid tubes. The oxygen factor was calculated by using the weights of carbon-dioxide, water, and the loss in weight of the respiratory chamber. The advantages of this method were that the readings could be made immediately after the bees were confined, and that large numbers of bees could be used in each experiment. Both workers and drones were used in measurements by this method.

The results obtained for each are given in figure 13, page 37. The first hour oxygen factor for worker bees was 780.0 cubic millimeters per bee per hour and that for drones was 577.0 cubic millimeters per bee per hour. The respiratory quotient in most of these experiments was greater than one (1). This would suggest almost complete carbohydrate metabolism.

Oxygen consumption and carbon-dioxide output in cubic millimeters per bee per hour at zero degrees Centigrade - 760 mm.

Oxygen = 299.5. Carbon-dioxide = 283.8. Respiratory Quotient = 0.947.

| OXYGEN | | | |
|---|---|---|---|
| Date | No. Bees | Temperature | Cu. mm. per hour |
| May 8, 1925 | 5 | 26.3 | 286.0 |
| " 8, " | 5 | 25.5 | 276.0 |
| " 9, " | 4 | 26.1 | 257.0 |
| " 9, " | 4 | 25.9 | 319.5 |
| " 9, " | 4 | 25.6 | 258.0 |
| " 21, " | 4 | 26.1 | 464.0 |
| " 21, " | 4 | 25.9 | 283.0 |
| " 21, " | 4 | 26.0 | 365.0 |
| " 22, " | 4 | 26.3 | 315.0 |
| " 22, " | 4 | 26.0 | 264.5 |
| " 22, " | 4 | 26.1 | 287.5 |
| June 16, " | 4 | 26.0 | 214.0 |
| " 20, " | 4 | 26.0 | 298.0 |
| " 23, " | 4 | 26.0 | 310.0 |
| AVERAGE | | | 299.9 |
| OXYGEN minus CARBON-DIOXIDE | | | |
| June 8, 1927 | 4 | 25.8 | 15.95 |
| " 9, " | 4 | 26.0 | 19.15 |
| " 9, " | 4 | 25.9 | 7.75 |
| " 9, " | 4 | 25.5 | 5.93 |
| " 16, " | 4 | 26.0 | 20.90 |
| " 20, " | 4 | 26.0 | 28.30 |
| " 22, " | 4 | 26.0 | 12.75 |
| " 23, " | 4 | 26.0 | 14.58 |
| AVERAGE | 4 | | 15.65 |

Figure 12.

Oxygen consumption and carbon-dioxide output in cubic millimeters per bee per hour at zero degrees Centigrade - 760 mm. Haldane and Pembrey gravimetric method.

| Date | Temp. | No. Bees | Time | Grams Oxygen | Grams C O2 | Cu.mm. $O_2$ per bee | Cu.mm. $CO_2$ per bee | R.Q. |
|---|---|---|---|---|---|---|---|---|
| WORKERS | | | | | | | | |
| April 3 | 21.25 | 200 | 1 hr. | 0.218 | 0.3534 | 762.0 | 898.0 | 1.179 |
| " 3 | 21.25 | 200 | 2d hr. | 0.2126 | 0.3420 | 744.0 | 872.0 | 1.172 |
| " 24 | 25.5 | 130 | 1 hr. | 0.1596 | 0.2836 | 858.0 | 1122.0 | 1.297 |
| " 23 | 26.5 | 126 | 1 hr. | 0.1656 | 0.2498 | 919.0 | 1008.0 | 1.097 |
| " 23 | 26.5 | 126 | 2d hr. | 0.0989 | 0.1572 | 547.0 | 635.0 | 1.1619 |
| " 29 | 26.0 | 147 | 1 hr. | 0.1790 | 0.2304 | 852.0 | 799.0 | 0.937 |
| TOTAL | | 929 | | | AVERAGE | 780.0 | 889.0 | |
| DRONES | | | | | | | | |
| June 5 | 26.0 | 59 | 1 hr. | 0.0743 | 0.1140 | 881.0 | 983.0 | 1.115 |
| " 22 | 28.0 | 126 | 3 hr. | 0.2382 | 0.3214 | 441.0 | 432.0 | 0.981 |
| " 24 | 23.0 | 104 | 4 hr. | 0.2855 | 0.4421 | 481.0 | 541.0 | 1.126 |
| " 26 | 24.0 | 69 | 5 hr. | 0.2488 | 0.3625 | 505.0 | 535.5 | 1.059 |
| | | | | | AVERAGE | 577.0 | 622.8 | |

Figure 13.

Brood rearing temperatures: Thirty-four degrees Centigrade should be a temperature normal to the adult bees, since this temperature is maintained in the brood nest thruout the brood rearing cycle. In an attempt to arrive at normal basal metabolism at this temperature, a series of experiments were conducted on newly emerged adults. These bees had taken but little food and were active only as they crawled about in the instruments. The results of these experiments are given by Figure 14, page 39. The oxygen consumption was 223.2 cubic millimeters per bee per hour. This figure is well below that found for active adults at any temperature above 0.3° Centigrade. Oxygen consumption of 223.2 cubic millimeters would seem to be the nearest approach to basal metabolism for active adults found in these experiments.

A variety of results were obtained in the use of active adults at 34° Centigrade because of the excessive activity of the bees in some experiments. In some cases the bees died from over-exertion before the second hour readings could be taken. The results obtained at this temperature were arbitrarily divided according to their apparent activity. Figure 15, page 40, gives the results for bees where their activity seemed to be normal or where they became inactive before the experiment was completed. The oxygen consumption in this case was 578.0 cubic millimeters per bee per hour. Figure 16, page 41, gives the results where the activity seemed to be excessive. The oxygen consumption was 1718.3 cubic millimeters per bee per hour. Because of the wide variations in results as shown in the above tables no definite conclusion can be made relative to the respiratory quotient. It seems to be high during the fore part of the experiment, decreasing toward the latter part as death approached some of the bees.

Oxygen consumption of newly emerged adults, in cubic millimeters per bee per hour at zero degrees Centigrade - 760 mm.

OXYGEN

| Date | No. Bees | Temperature | No. Hours | Cu. mm. per hr. |
|---|---|---|---|---|
| May 5, 1927 | 1 | 34.0 | 3 | 278.1 |
| " 6, " | 1 | 33.8 | 1 | 198.4 |
| " 6, " | 1 | 33.8 | 1 | 268.4 |
| " 6, " | 1 | 33.0 | 1 | 118.0 |
| " 6, " | 4 | 33.8 | 1 | 222.0 |
| " 6, " | 4 | 33.8 | 1 | 279.5 |
| " 6, " | 4 | 33.0 | 1 | 199.2 |
| TOTAL | 16 | | 9 | 223.2 |

Figure 14.

Metabolic results per bee per hour at zero degrees Centigrade - 760 mm.

Normal activity.

| Date | No. Bees | Temperature | Cu.mm.per hr. | Activity |
|---|---|---|---|---|
| **OXYGEN** | | | | |
| April 22, 1927 | 2 | 34.0 | 724.0 | Normal or less |
| " 22, " | 2 | 34.5 | 433.0 | " " " |
| " 22, " | 2 | 34.0 | 692.0 | " " " |
| " 22, " | 2 | 34.5 | 532.0 | " " " |
| " 27, " | 2 | 33.8 | 485.0 | " " " |
| " 27, " | 2 | 33.8 | 624.0 | " " " |
| " 28, " | 2 | 35.5 | 554.0 | " " " |
| " 29, " | 2 | 33.8 | 403.0 | " " " |
| " 30, " | 2 | 35.0 | 634.0 | " " " |
| " 30, " | 2 | 34.2 | 698.0 | " " " |
| AVERAGE | | | 578.0 | |
| **OXYGEN minus CARBON DIOXIDE** | | | | |
| April 19, 1927 | 4 | 34.0 | 97.7 | Normal or less |
| " 19, " | 4 | 34.0 | 87.6 | " " " |
| " 20, " | 4 | 34.0 | 79.5 | " " " |
| " 21, " | 4 | 33.8 | 69.4 | " " " |
| " 21, " | 4 | 33.8 | 82.0 | " " " |
| AVERAGE | | | 82.2 | |

Figure 15.

Metabolic results per bee per hour at zero degrees Centigrade - 760 mm. High activity.

| Date | No. Bees | Temperature | Cu.mm.per hr. | Activity |
|---|---|---|---|---|
| **OXYGEN** | | | | |
| April 27, 1927 | 2 | 33.8 | 1738.0 | Very active |
| " 28, " | 2 | 35.5 | 1074.0 | " " |
| " 29, " | 2 | 33.8 | 1712.0 | " " |
| May 4, " | 2 | 33.5 | 1950.0 | " " |
| " 4, " | 2 | 33.5 | 2120.0 | " " |
| AVERAGE | | | 1718.8 | |
| **OXYGEN minus CARBON DIOXIDE** | | | | |
| April 21, 1927 | 2 | 34.0 | 135.2 | " " |
| " 20, " | 2 | 34.0 | 103.0 | " " |
| " 21, " | 2 | 34.0 | 147.0 | " " |
| AVERAGE | | | 128.4 | |

Figure 16.

Oxygen consumption and carbon dioxide output in cubic millimeters per bee per hour at zero degrees Centigrade.

Oxygen = 462.8. Carbon-dioxide = 445.7. Respiratory Quotient = 0.942.

| Date | No. Bees | Temperature | Cu. mm. per hr. |
|---|---|---|---|
| OXYGEN | | | |
| May 22, 1925 | 4 | 35.5 | 662.5 |
| " 23, " | 4 | 35.6 | 618.0 |
| " 23, " | 4 | 35.9 | 394.0 |
| " 23, " | 4 | 35.8 | 277.0 |
| AVERAGE | | | 462.8 |
| OXYGEN minus CARBON DIOXIDE | | | |
| June 4, 1925 | 4 | 35.7 | 3.54 |
| " 4, " | 4 | 35.7 | 17.70 |
| " 4, " | 4 | 35.6 | 34.50 |
| " 4, " | 4 | 35.8 | 12.60 |
| AVERAGE | | | 17.08 |

Figure 17.

Figure 17, page 42, gives results for adults at 36° Centigrade. Nothing unusual in activity or condition was noted, so assuming that it was normal for high temperatures, the oxygen factor was 462.8 cubic millimeters per bee per hour, carbon dioxide 445.7 cubic millimeters per bee per hour, and the respiratory quotient was 0.94 . The number of experiments at this temperature is not sufficient to draw any definite conclusions.

Metabolism as influenced by temperature range: The graph of data for all temperatures used is given in Figure 18, page 44. This curve is complete only in so far as my experiments are concerned and can be assumed to be true only for the conditions under which the experiments were carried out. A true metabolic curve will be established only by additional experiments for all temperatures thruout the yearly cycle.

Reaction of bees to experimental conditions: As has been stated previously, the adult bees reacted in a different manner to every set of experimental conditions. A graphical representation of these reactions is given in Figure 19, page 45. It will be noticed that temperature as related to activity had an important bearing on the life of a bee during the experiments. The higher temperatures seemed to shorten the lives of the bees proportionally to the amount of food they carried at the time they entered into the experiment. The arrows show the apparent duration of life as indicated by the results secured at the respective temperatures.

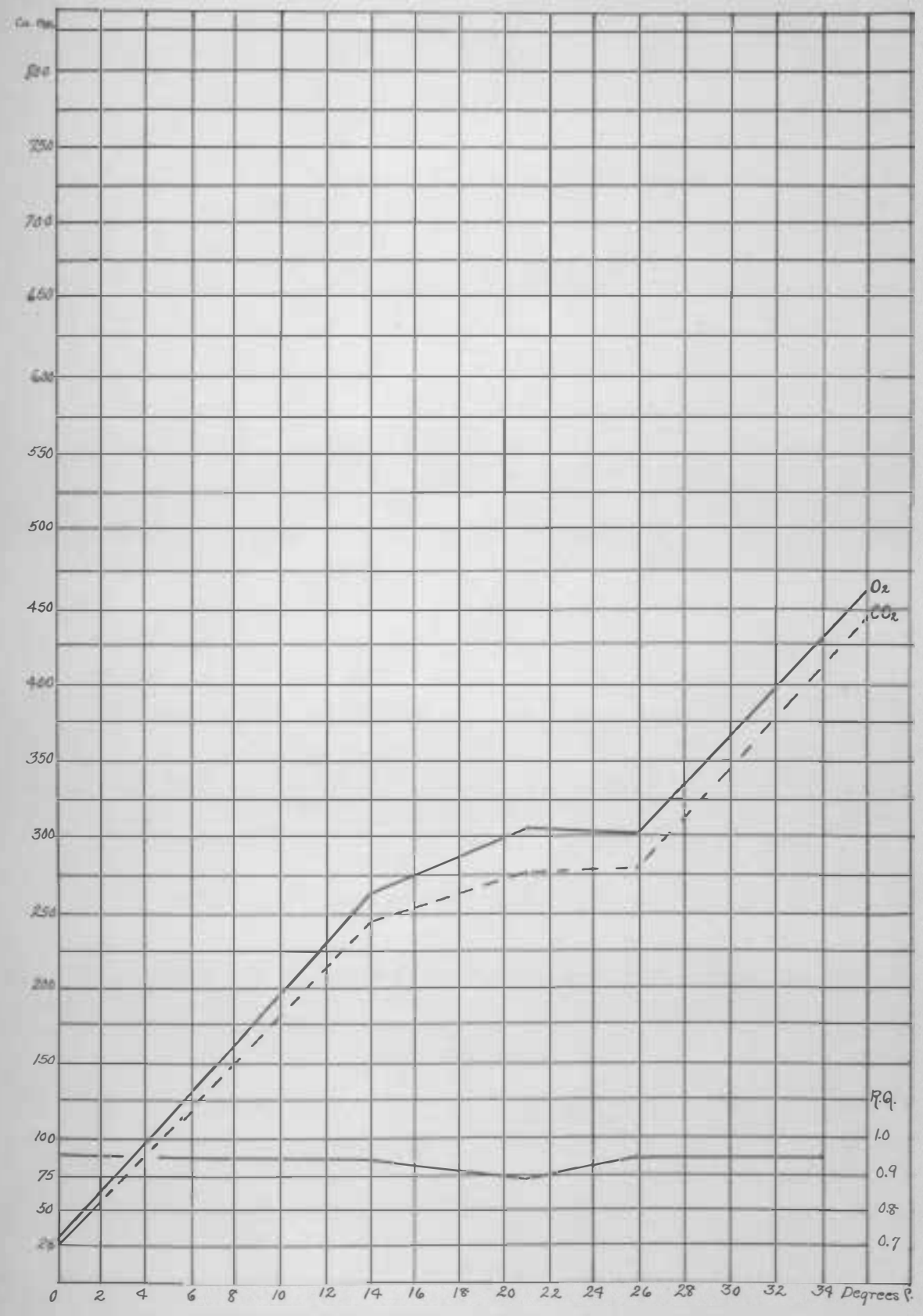



FIGURE 18 HONEY BEE METABOLISM ACCORDING TO TEMPERATURE 2d HOUR

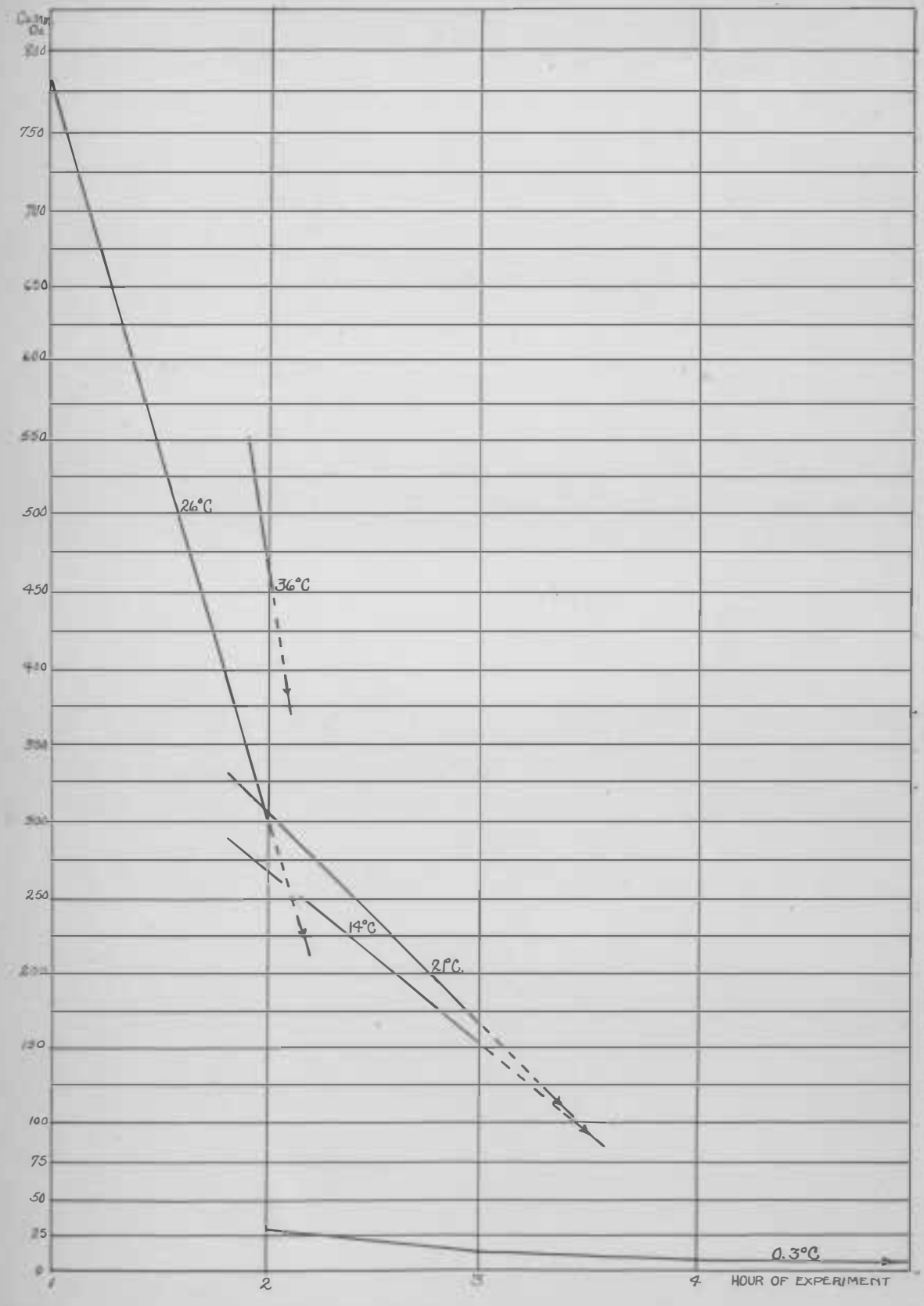



FIGURE 19 REACTION OF BEES TO EXPERIMENTAL CONDITIONS

Deductions: An interesting deduction can be made from the results of these experiments as to the amount of oxygen consumed as related to body weight. According to Park, the minimum flying weight of a worker bee is 83.0 milligrams. One kilogram of bees would, therefore, contain 12,195 bees. Reducing the number of cubic millimeters of oxygen used by the 12,195 bees at the various temperatures to liters by the formula, $\frac{12,195.0 \times \text{Oxygen in cu. mm.}}{1,000,000}$, we derive the liters of oxygen per hour consumed by a kilogram of honey bees. The complete data for this computation is shown by Figure 20, page 47. It will be noted that a minimum use occurs at 0.3° Centigrade, where 0.36 liters were consumed per kilogram. At 14° Centigrade, 21° C. and 26° C., between 3.23 and 3.67 liters per kilogram were used; at 34° C. (moderate activity) 7.05 liters were required; and 20.96 liters per kilogram were used in excessive activity. The metabolism for bees at 0.3° C. only, approaches the oxygen consumption of man per kilogram of body weight, for the higher temperatures show a much higher rate.

Heat Production: According to previous calculations in the metabolism of honey, each liter of oxygen consumed produced 4.368 large calories of heat. Knowing the number of liters of oxygen used hourly per kilogram of bees, the amount of heat generated at the various temperatures can be calculated. The minimum heat production works out to be 1.57 plus calories per kilogram at 0.3 degrees Centigrade, and a maximum of 7.54 plus calories per kilogram at 34 degrees Centigrade (excessive activity). Such calculations are necessarily true only for bees under experimental conditions, and would not necessarily be true for bees under hive conditions. Within the hive where activity would be reduced to the minimum, the heat produced would be held at a minimum. The minimum heat factor would be much below that found for bees under experimental conditions.

Liters of oxygen used per kilogram of bees (minimum flying weight of 82 milligrams) for the second hour after exposure at the respective temperatures.

| Temperature | Cu. mm. per hr. Oxygen | Liters Oxygen | Activity |
|---|---|---|---|
| 0.3°C. | 29.64 | 0.36 | None |
| 14.0°C. | 265.2 | 3.23 | Moderate |
| 21.0°C. | 308.0 | 3.76 | " |
| 26.0°C. | 299.9 | 3.67 | " |
| 34.0°C. | 578.0 | 7.05 | " |
| 36.0°C. | 462.8 | 5.64 | " |
| 34.0°C. | 1718.8 | 20.96 | Excessive |

Figure 20.

Conclusions: At the temperature of 0.3° Centigrade, the adult honey bees became inactive almost immediately and their metabolic rate decreased rapidly for about six hours, after which the decrease continued very gradually. The bees recovered, when returned to room temperature, after having been subjected to 0.3°C. for a period of twenty-four hours or longer, existing during this time at a very low rate of metabolism.

Since there was no apparent activity at 0.3°C., and seemingly normal activity (for experimental conditions) at 14.0°C., the great increase in the metabolic rate over that range in temperature indicates that activity is a determining factor in the rate of body metabolism.

Evidently the activity within the hive at 14.0°C. is considerably less than that for bees under experimental conditions at that temperature. If the metabolic rate of bees within the hive were as great as that of bees under these experimental conditions, they could not exist over winter on the supply of food stored in the hive. Under normal winter conditions there must be a specially organized metabolic rate, thru cluster formation, in order to conserve the food supply of the colony.

Experiments conducted at the temperatures of 14.0°C., 21.0°C., and 26.0°C. indicate that over this range of temperature adult honey bees are but little influenced by the temperature variations.

The adult honey bees were unusually active at 34.0°C. and 36.0°C. This excessive activity caused death in a very short time and the length of life under these experimental conditions was proportional to the amount of honey each bee carried. Experiments indicate that

adult honey bee metabolism is greatly variable at these temperatures.

The respiratory quotients for all temperatures show that the diet of the adult honey bee is composed mainly of carbohydrates.

The removal of adult bees from their normal surroundings seemed to create an abnormal amount of activity measurable directly in the rate of metabolism.

The rate of metabolism of adult honey bees is influenced to some extent by the temperature.

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