THE RESPIRATORY CURRENTS IN THE TRACHEAL SYSTEM OF THE ADULT HONEY-BEE

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INTRODUCTION

Interest in the respiration of the bee has been aroused on several occasions in the past, largely because of the disease caused by the infestation of the tracheae of the first thoracic spiracles by the mite *Acarapis woodi* (Rennie).

White (1921) investigated the pathological effects on the bee of blocking the anterior spiracles with wax. He found that such bees lost the power of flight at once and he related this to the similar effects of severe infestation by *A. woodi*.

Morison (1927) put powdered charcoal in the depression in the cuticle close to the aperture of the anterior spiracles and noted that the charcoal was alternately blown away and drawn towards the spiracular opening. Since the powder was only feebly drawn towards the opening Morison concluded that a greater volume of air left the spiracle than entered it, and that there was a circulation of air from the posterior regions of the body of the bee to the anterior spiracle.

Wohlgemuth (1929) separated the abdomen and thorax of the bee with a rubber diaphragm and was able to anaesthetize the insect by putting either the thorax or abdomen into chloroform vapour. He concluded that inspiration took place by both abdominal and thoracic tracheae. He also examined the flow of air near the spiracular openings by holding a needle covered with minute soot particles near the openings and observing the movements of the particles. He finally concluded that the bee inspired through all spiracles during abdominal expansion and that expiration occurred through the thoracic spiracles only.

Sachs (1952) was able to show that individuals of *A. woodi*, when placed on the thorax of the bee, were attracted towards the first spiracle. By supplying an artificial current of air to the first spiracles of the empty thoraces of dead bees he found that the mite was attracted to the opening by intermittent puffs of air of about 120 per minute.

Connell & Glynne-Jones (1953) examined the respiratory activity of bees by (*a*) using a modification of the apparatus used by Fraenkel (1932), (*b*) high-speed photography, and (*c*) observing the entry of minute dust particles into the spiracles. They concluded that both thoracic and abdominal spiracles serve for expiration and inspiration, and that a current of air flowing from abdomen to thorax or vice versa was unlikely.

The following paper describes further observations upon the respiratory currents in the tracheae of bees. It has been found that the air circulation is rather more complex than has previously been supposed.

METHODS

The activity of the propodeal ('third thoracic') spiracles was observed directly, with the bee lightly clamped under a binocular microscope. The activity of the other spiracles cannot be seen. The thoracic spiracles are hidden under folds of the cuticle, and the abdominal spiracles open into cavities which then lead to a passive external opening. (Snodgrass (1925) and Wohlgemuth (1929) give full descriptions of the anatomy of the spiracles.)

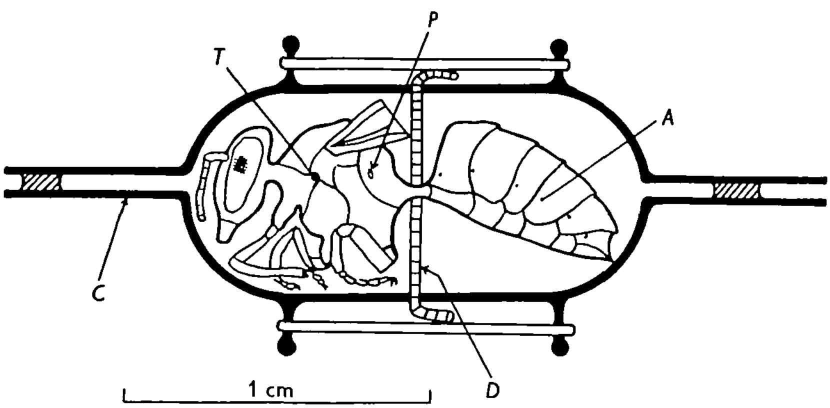

Fig. 1. Diagram of the apparatus used with the bee in the gas chamber. *C*, capillary tube leading from main chamber; *D*, rubber diaphragm; *A*, one of the six pairs of external openings of the abdominal spiracles; *P*, propodeal ('third thoracic') spiracle; *T*, lobe of body wall shielding first thoracic spiracle.

Fraenkel's technique (1932) was used to observe the air currents passing through the body of the bee. A gas chamber was made from glass tubing of 6 mm. internal diameter. The chamber was about 2 cm. long and both ends were drawn out into fine capillaries of about 0·5 mm. internal diameter. A hole was burned into a thin rubber diaphragm and the diameter of the hole was made approximately the same as the diameter of the petiole of the bee. The bee was fixed in the diaphragm and a small drop of water was spread on to the petiole, thereby sealing the joint between it and the rubber. The rubber was held firmly between the ground ends of the two halves of the chamber with the thorax and head of the bee in one half and the abdomen in the other half. Drops of water were introduced into each capillary tube. The wings of the bee were clipped short before it was placed in the chamber (Fig. 1).

For some experiments the first thoracic spiracles or the propodeal spiracles were blocked with beeswax in the following manner. The bee was anaesthetized with CO_2 and clamped lightly under a binocular microscope; a piece of wax was held on the spiracle with a fine needle and the needle was then touched with a hot wire. The wax melted over the spiracle and the needle was quickly withdrawn.

The second thoracic spiracles were ignored since their openings are exceedingly minute relative to those of the other spiracles.

RESULTS

A. *The action of the propodeal spiracles*

Usually the spiracles were closed, but occasionally they opened and closed with a rhythm which appeared to be synchronous with abdominal pumping; such movements were rapid and of brief duration.

The spiracular mechanism was very sensitive to carbon dioxide; the direction of a stream of carbon dioxide on to a bee whose third spiracle was closed caused the latter to begin rhythmical movements immediately. By this means it was established that the spiracle was open when the abdomen was contracting and closed when the abdomen was expanding. Exposure to a high CO_2 concentration anaesthetized the bee and the spiracle again became permanently closed whilst the bee was immobile.

B. *The movement of air in the chamber*

(*a*) *Normal bees*

(i) *In normal air.* The drops of water in the capillary tubes began oscillating as soon as the bee was enclosed in the chamber. Their movements were approximately 1 cm. each way, synchronized and in the same direction. The drop in the abdominal capillary tube approached the bee as the abdomen contracted and receded as the abdomen expanded. The drop in the thoracic tube receded from the bee as the abdomen contracted and approached as the abdomen expanded. The frequency of this movement was between 100 and 200 cyc./min.

The centre of oscillation of the drops usually moved slowly along the tubes (about 5 mm. in about 10–30 sec.) as a result of the transference of air from one half of the chamber to the other. The flow was usually from thorax to abdomen but the direction of this slow airstream was variable.

(ii) *In air plus* CO_2. A bee was allowed to feed on strong syrup and was then placed in the chamber. It was allowed to settle down for some minutes with the two halves of the chamber left ajar. The halves were then closed upon each other. Strong oscillating movements of the drops occurred with a superimposed migration showing a slow air flow from thorax to abdomen.

CO_2 was then blown into both sides of the chamber; the slow air flow immediately reversed direction but the superimposed oscillations continued. The chamber was then opened, aired for a minute or two, and then closed; the air flow now returned to its original direction. This experiment was repeated several times. The drop of water in the abdominal capillary tube was then removed and the bee was left undisturbed in the chamber; the drop of water in the thoracic tube was drawn towards the head and eventually it spread out and burst as the tube widened into the chamber. The water then ran back into the narrow tube and repeated the process. The bee was left overnight and next day the drop of water was still moving towards the head and bursting. CO_2 was then blown into the chamber and again there was an immediate and definite reversal of this air flow.

From the results of the experiments described above it appears that a tidal flow of air normally passes in and out of the thoracic spiracles and this flow is caused by the pumping movements of the abdomen.

The superimposed slow current of air was undoubtedly regulated by the activities of the spiracles. In air plus CO_2 the abdominal spiracles must have been relatively closed compared with the thoracic spiracles when the abdomen was contracting and relatively open when the abdomen was expanding. Thus a movement of air from abdomen to thorax was created.

The observed activity of the propodeal spiracle in air plus CO_2 was consistent with this interpretation. However, in air plus CO_2, air must have passed into the thoracic spiracles to some extent when the abdomen was expanding, otherwise there would have been no oscillation of the water drops.

(*b*) *Bees with blocked propodeal spiracles*

(i) *In normal air*. The behaviour of the water drops in the capillary tubes seemed to be exactly as before. Strong oscillation of the same amplitude occurred and overall migration of the drops was usually from thorax to abdomen but occasionally this was reversed.

(ii) *In air plus* CO_2. The air current was now rapid and its direction was consistently from thorax to abdomen, but the strong oscillations of the drops continued.

It now seemed that the first thoracic spiracles were also affected by CO_2, but their opening and closing movements, although still synchronized with abdominal pumping, were in opposition to those of the propodeal spiracles. Thus they were open when the abdomen was expanding and were closed relative to the abdominal spiracles when the abdomen was contracting in air and CO_2, thereby creating a flow of air from thorax to abdomen.

They could not have closed completely, however, since the oscillation of the water drops continued and some air must therefore have been passing out of the partly closed spiracles when the abdomen was contracting.

(*c*) *Bees with blocked first thoracic spiracles*

(i) *In normal air*. There was hardly any movement of the drops, the oscillation being no more than 1 or 2 mm., with no detectable directed airflow.

(ii) *In air plus* CO_2. There was an extremely rapid movement of air from the abdominal chamber to the thoracic one and there was no oscillation. The drops of water moved along in jerks synchronous with the contraction of the abdomen.

Since the anterior spiracles were blocked and the propodeal spiracles were closed there was neither a tidal oscillation nor an air stream in normal air.

In air plus CO_2 air was pumped efficiently from the abdomen through the propodeal spiracles. The lack of oscillation in normal air and air plus CO_2 meant that no air could pass into the propodeal spiracle (or the second thoracic spiracle) as the abdomen expanded. The tidal oscillation occurring under normal conditions must therefore take place via the first thoracic spiracle.

DISCUSSION

It was concluded from these results that the inactive bee inhales and exhales via the first thoracic spiracles, the air movement being created by the pumping movements of the abdomen. The flying bee probably changes to the other condition when the spiracles come into action under the stimulus of metabolic CO_2 production. Air would then be inhaled at the first spiracle and largely exhaled via the propodeal spiracle. Thus a rapid flow of fresh air would pass through the thorax of the insect providing thorough ventilation in a region of relatively high carbon dioxide production.*

The inability of bees to fly when heavily infested with *Acarapis woodi* in the tracheae leading from the first spiracles, or when the latter are artificially blocked, is therefore understandable.

Movement of air through the abdominal spiracles is slight under low CO_2 tensions, but a current of air probably flows into them and out of the propodeal spiracle under high CO_2 tensions.

SUMMARY

1. The propodeal spiracles of the honey-bee remain closed in normal air but in air plus CO_2 they open as the abdomen contracts, and close as the abdomen expands.

2. The first thoracic spiracles remain open in normal air but in air plus CO_2 they nearly close as the abdomen contracts, and open as the abdomen expands.

3. When the bee is in normal air there is a strong tidal flow of air in and out of the first thoracic spiracles, but only a weak and variably directed stream of air through the body.

4. It is deduced that when an active bee is producing metabolic CO_2, directed streams of air enter the first thoracic spiracles and the abdominal spiracles and leave via the propodeal spiracles.

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* This behaviour of the spiracles is not likely to be dependent directly upon oxygen lack since Mr J. Simpson has shown that in an atmosphere which is 45 % CO_2 the bee becomes completely motionless, but oxygen tension must be lower than 7 % before a perceptible change in the activity of the bee can be seen.