

Zoology. — “*On a new form of breathing regulation (regulation of diffusion) in Insects and Arachnida.*” By E. H. HAZELHOFF.
(Communicated by Prof. R. MAGNUS.)

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Several investigators have described respiratory movements in a great number of Tracheata, corresponding in principal with those of Mammals and whereby an air-current is alternately drawn into and expelled from the respiratory organs.

Although it was expected, no trace of respiratory movements could be found in many Tracheata (Myriapoda, Arachnida, almost all Insect larvae and all pupae) even when highly magnified. WINTERSTEIN for instance says in his „Handbuch der vergleichenden Physiologie“, Band I, 2, pag. 111—112, that when one considers how exceptionally fine the extreme ramifications of the tracheal system are, one can hardly imagine how the tissues can receive a sufficient supply of oxygen. According to WINTERSTEIN this applies to both Tracheata with and without respiratory movements.

On page 113 WINTERSTEIN concludes: „Das Gesagte wird hinreichen, um zu zeigen, wie weit wir..... bei der Respiration der..... Insekten von einem Verständnis des Mechanismus ihres Gaswechsels in Wahrheit entfernt sind“.

A great improvement in this unsatisfactory state of the problem was caused by a short but very important paper by KROGH (A. KROGH. Studien über Tracheenrespiration II. Ueber Gasdiffusion in den Tracheen. Pflügers Archiv Band 179. 1920 pag. 95—112).

For a few Tracheata KROGH determines the normal amount of oxygen used. He then takes the average length and width of the tracheae and on the basis of these data he can now calculate, that an oxygen pressure difference of only a small percentage of one atmosphere is sufficient to cause inward diffusion of the required amount of oxygen.

In the Myriapod *Scutigera* e.g. an oxygen pressure head of 0.13 % of one atmosphere is sufficient (therefore an oxygen pressure of 20.93 %—0.13 % = 20.8 % in the extreme ramifications of the tracheal system); in the larva of *Cossus ligniperda* a diffusion head of 1.7 %; in the larva of *Tenebrio molitor* 2.2 %; and in the larva of *Lasiocampa* 1.7 %.

From these data the conclusion may be drawn that in many neither too large nor too active insects respiratory movements are absolutely superfluous as diffusion alone is quite sufficient to provide the tissues with oxygen. In very large and active Insects respiratory movements do occur.

It seems that former investigators have not realised sufficiently how

exceptionally short the distances are in which diffusion must take place, and that consequently the effect of diffusion has been underestimated.

In his calculations KROGH has not reckoned with the existence of tracheal valves near the stigmata. In the scarce and very scattered literature about the movements of these tracheal valves I found that these movements can be seen distinctly in several Orthoptera, owing to the fact that here the tracheal valves are joined to the stigmata. (In many other Insects the tracheal valves lie further from the stigmata so that their movements cannot be seen from the outside.)

In my investigations on *Periplaneta americana* it soon became evident that the first and second pairs of thoracic stigmata are mostly closed (or at least almost closed) when the insect does not move. Without hurting it, the insect was fastened to a corkplate by means of pins and placed in a trough closed hermetically with a cover of plate glass. An air-current (and afterwards also other gasmixtures) was drawn through the trough.

The fixing of the insect in a definite position is necessary as otherwise the stigmata are generally invisible.

Through a binocular microscope (magnification 10 or 20 times) the movements of the above named stigmata, especially those of the well developed first pair, are distinctly visible. The experiments were carried out at a constant temperature; usually at 28° C.

At a temperature of 28° C. *Periplaneta americana* shows no respiratory movements when at rest. The first two large thoracic stigmata mostly show very quick irregular oscillations (sometimes a few per second), whereby the opening of the stigmata generally remains very narrow, varying between 0 and $\frac{1}{10}$ or $\frac{1}{4}$ of the maximum opening¹⁾. When the insect moves the stigmata immediately open widely, and the quick oscillations cease. After the movements the stigmata still remain open for a short time (e.g. $\frac{1}{2}$ minute); then the quick oscillations of the stigmata reappear and the width of the opening gradually diminishes till it finally reaches its original state.

Apparently this must be considered as a peculiar form of breathing regulation, whereby the insect remains nearly passive. After an increase in the metabolism the intake of oxygen and output of carbon dioxide are increased by the enlargement of the stigmata (i. e. by improvement of the diffusion conditions), and not as could be expected by the appearance of real breathing movements. These movements do occur but only when the insect has been very active. The animal reacts on a lack of oxygen (respectively on too much of carbon dioxide) firstly by opening the stigmata and sometimes also by executing respiratory movements.

To determine exactly which stimulus causes the opening of the stigmata, I investigated the effect of different gas-mixtures, with a higher

¹⁾ At 12° C. the thoracic stigmata are closed or almost closed and the quick oscillations have ceased.

percentage of carbon dioxide or a lower percentage of oxygen than air.

It soon became evident that in a mixture with a low percentage of carbon dioxide (e.g. 2%) the stigmata open widely.

It was remarkable that a gas-mixture with only 10% oxygen (and 90 % nitrogen) never produced any effect; the insects were therefore not noticeably influenced by a considerable decrease of the oxygen pressure (a very low percentage of oxygen, e. g. 5 % and lower, really does cause the opening of the stigmata but at the same time also respiratory movements appear; a carbon dioxide pressure of 12 % or more produces the same effect).

We must therefore conclude, that the opening of the stigmata after the insect has moved is the result of the increased carbon dioxide pressure either in the tissues, or in the tracheae.

Furthermore I tried to determine if *Periplaneta* reacts on a increased carbon dioxide pressure in the tissues or on a increased carbon dioxide pressure in the neighbourhood of the stigmata.

A *Periplaneta* was fastened to a movable corkplate which was mounted in such a way, that with a slight movement the right or the left foremost thoracic stigma could be brought in the field of the binocular microscope. With the help of a fine glass capillary an air-current was directed towards the left stigma while from a second capillary a gas-current consisting of 5 % carbon dioxide and 95 % air was directed towards the right. Both stigmata apparently reacted quite independently of each other; the left stigma was closed or almost closed and the right wide open. When the 5 % carbon dioxide current on the right stigma was interrupted it closed after a short time (generally after one minute). When the 5 % CO₂ current was started again, the stigma reopened shortly afterwards (e. g. after 5 sec.; in one experiment the stigma reopened several times after 1 or 2 sec.). In these experiments the air-current was directed uninterruptedly towards the left stigma, and this stigma indeed remained closed or almost closed.

I now tried the effect of an interrupted air-current on the left stigma, while an uninterrupted 5 % carbon dioxide current was continuously flowing towards the right. The right stigma remained open, the left one opened 1/2 to 2 minutes after the air-current was shut off and closed 1 to 5 sec. after the current was started again. Finally the same experiments were taken with the reversed, i. e. an air-current flowing towards the right stigma and a 5 % carbon dioxide current towards the left. The results quite agreed with what could be expected from the above.

All these results are satisfactorily explained if we suppose that the two foremost thoracic stigmata react absolutely independently of each other on the carbon dioxide pressure in their immediate neighbourhood. Apparently we are dealing with a peripheral regulation; from the very short reaction time it is obvious that the perception must take place near the stigma. In connection with this one is inclined to think of the sensory

hairs which were found by Alt (*Zeitschrift für wissenschaftliche Zoologie*, Band 99, 1912, pag. 378 en 379) on the thoracic stigmata of *Dytiscus marginalis*.

The phenomenon that stigmata, which are almost closed in the air, open in a gas-mixture with a low percentage of carbon dioxide, was furthermore observed in the book-lungs of three different Arachnida, namely: *Epeira diademata*, *Meta segmentata* and *Tetragnatha obtusa*. A peculiar difference between *Periplaneta* on the one hand and the three above mentioned Arachnida on the other hand is, that in Arachnida I never observed quick oscillations of the stigma. The experiments showed further, that in Arachnida a distinct *quantitative* relation exists between the carbon dioxide pressure of the surrounding air and the width of the stigma opening: in 1 % carbon dioxide the stigmata open slightly; in 1½, 2, 2½ and 4 % carbon dioxide they open more and more; in 5 % and especially in 8 or 10 % carbon dioxide the stigmata open very widely.

As in *Periplaneta* a decrease in the oxygen pressure to 10 % and even to 5 % of one atmosphere has no effect.

V. WILLEM (Observations sur la circulation sanguine et la respiration pulmonaire chez les Araignées. *Arch. Néerl. de Physiol. de l'homme et des animaux*. Tome I pag. 226. 1917) observed quick expansions and contractions of the complex of laminae of the book-lung of *Pholcus* synchronous with the beatings of the heart, and therefore in all probability caused by variations of the blood-pressure. The air-currents which, according to WILLEM, should be the result of these expansions and contractions, could however not be demonstrated (l.c. pag. 253).

On the basis of the quoted paper by KROGH and on the basis of my own investigations regarding the varying width of the stigma opening I think it probable that also in Arachnida diffusion is the most important process. One particular muscle which by its contraction causes a widening of the vestibulum of the book-lung, and which is considered by WILLEM as an inspiratory muscle, must, according to my opinion, be considered as the muscle which enlarges the width of the stigma opening in order to increase the speed of diffusion. I am of this opinion, because we see that at a low percentage of carbon dioxide this muscle does not contract repeatedly but only once, the stigma remaining open.

Further investigations regarding these problems are in course of preparation.

From the above it follows that the foremost thoracic stigmata of *Periplaneta* and also the stigmata of the book-lungs of different Arachnida are generally almost closed when the animal is at rest; a definite stimulus (a low percentage of carbon dioxide) is necessary to open them.

It is evident that when the stigmata are closed the conditions of diffusion must be less favourable than when they are open. When, therefore, the

stigmata after having been open for a long time are closed at a certain moment, then the giving off of carbon dioxide from the stigmata is of course at first diminished ; but as the production of carbon dioxide in the tissues continues with exactly the same speed, the carbon dioxide pressure will be a somewhat increased after a short time in the whole tracheal system, and the carbon dioxide pressure difference between the air just inside and the air just outside the almost closed stigma will soon have risen to such an extent, that the carbon dioxide delivery from the stigma will resume its original velocity.

In other words : when a stigma almost closes, the output of carbon dioxide from the tracheae will at first be decreased, but will afterwards resume its original speed. And vice versa : by the opening of a stigma that has been closed for some time, the output of carbon dioxide from the tracheae will at first be increased but after some time will resume its original velocity also in this case. The above is also applicable to the intake of oxygen.

In all probability the same is not applicable to the output of water vapour from the tracheae. While probably the production of carbon dioxide in the tissues is not dependent on the carbon dioxide pressure, the production of water vapour in the finest ramifications of the tracheal system is on the contrary highly dependent on the amount of water vapour already present. The closing of the stigmata after having been open a long time will consequently cause an increase in the relative humidity of the air in the tracheal system, and accordingly the production of new quantities of water vapour will be hampered ; so we must conclude that when the stigmata are almost closed, the output of carbon dioxide will remain the same in the long run while the output of water vapour will decrease.

I think it not improbable that a decreased output of water vapour is of great importance especially to those Tracheata living in a dry atmosphere, because in this way a drying up of the tissues could be prevented.

S u m m a r y.

In the examined Tracheata the tracheae and book-lungs are generally almost closed. The stigmata are open only in so far as the exchange of the gases of respiration demands. A carbon dioxide pressure of only 2% is sufficient to cause the opening of the stigmata.

The two foremost thoracic stigmata of *Periplaneta* react independently of each other on the carbon dioxide pressure in their immediate neighbourhood.

One could call it a kind of breathing regulation, not however a regulation of the respiratory movements but a regulation of diffusion.

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