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INVESTIGATION OF TRACHEAL RESPIRATION II:
ON GASEOUS DIFFUSION IN THE TRACHEA

[Following is a translation of an article by August Krogh, of the Zoophysiological Laboratory of Kopenhagen, received 3 September 1919, in the German-language publication, Pflugers Arch. Ges. Physiol., Vol 179, 1920, pp. 95-120.]

(Note: The first communication of this series, "On the Composition of the Air in the Tracheal System of Some Insects," was published in Skand. Arch. Physiol., vol. 29, 1913.)

We know that the tracheal system of the insects consists of chitin-coated tubes which start from a number of openings in the outer skin -- spiracles or stigmata -- become greatly ramified and terminate in the various organs of the bodies with very numerous and extremely delicate ramifications. According to the investigations of Cajal, Holmgren and Wielowiejski (Note: Cajal, Zeitschr. wiss. Mikrosk., 7, 1890; Holmgren Festschr., f.W. Liljeborg Upsala, 1896; Wielowiejski, Zeitschr. wiss. Zool., 37, 1882) the thinnest branchlets of the chitinous tracheae become transformed into a dense network of even finer air channels (estimated by Cajal at a maximum of 0.2 μ in diameter) which penetrate the cells and envelop, for instance, all muscle fibrils. The chitinous tracheae with which we shall occupy ourselves exclusively in the following, usually are of circular cross sections and equipped with a spiral reinforcing ridge -- the spiral thread. This structure as well as the small dimensions in general of the tracheae offer a very considerable resistance to pressure so that a relatively voluminous mechanical ventilation of the tracheal system can hardly be produced. On the other hand, it is difficult to say how an incomplete compression of the tracheal system could effect a renewal of the air in the delicate terminal branches and we must admit that a satisfactory explanation of the respiratory mechanism of the tracheata is so far lacking.

For example, Winterstein writes (Note: Handbook of Comparative Physiology, Vol I, 2, Jena, 1912, pp. 111-112), "Among the greater number of tracheata, the renewal of the respiratory air is effected by special

respiratory motions....we intend here merely to point out the difficulties which confront the understanding of the mechanism of an adequate renewal of the air even where such respiratory motions have been observed (insects), if we consider that, in spite of the relatively perfected respiratory mechanism, in an organ of such easily and extensively variable volume as the mammalian lung, the air in the alveoli differs both in its oxygen as well as in its carbon-dioxide content by several percent from that of the exterior air, it then seems scarcely conceivable how a sufficient renewal of the gas content can be accomplished even by very extensive respiratory motions in a system of thin and relatively rigid tubes in which the thinnest terminations are close to the border of that dimension observable under the microscope."

Moreover, in many tracheata, special respiratory motion cannot be demonstrated and the widely held assumption that movements of locomotion, etc. can effect sufficient ventilation is certainly not valid. We should here also point out the closed tracheal systems in water insects equipped with tracheal gills. Among most of them, no such mechanisms are known which would be able to effect a gaseous exchange between the tracheal gills and the remainder of the tracheal system.

These difficulties induced me to consider a quantitative investigation on how much of a gaseous exchange might be accomplished by simple gaseous diffusion along the tracheae. From the beginning, it appeared to me as rather improbable that sufficient ventilation could be produced in this manner because I was aware from numerous gas-analytical experiences how slowly diffusion flow takes place, but I considered it possible that diffusion might be an appreciable auxiliary aid.

The processes of gaseous diffusion can be illustrated by means of Fig. 1. A is a tube with a length of 1 cm and a cross sectional surface of a cm². In container B takes place a continuous process consuming S cm oxygen per second. To simplify matters, we shall temporarily disregard the formation of carbon dioxide which takes place simultaneously in the organism. The gaseous molecules all move in a

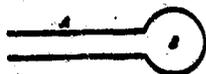


Fig. 1

straight line but continuously change their direction due to the numerous collisions of the molecules. If the oxygen molecules are distributed uniformly, the same number of oxygen molecules per unit time will pass through a given cross section of the tube in both direction; diffusion is in equilibrium. However, if we assume that oxygen is consumed at B, the equilibrium becomes unbalanced and a lesser number of oxygen molecules will move from B to A than from A to B; oxygen will diffuse into B. This diffusion will take place as much more quickly as the number of oxygen molecules in B decreases and there will finally occur in B a state of equilibrium in which the consumption of oxygen is precisely equalized by diffusion. The oxygen pressure in the atmosphere shall be assumed to be p_1 and p in B (p and p_1 expressed by standard atmospheric pressure as unit. We then have

$$s = \frac{(p_1 - p) \cdot c}{l}$$

i.e. the inflow of oxygen per second is proportional to the differential pressure ($p-p_1$) and the cross section a , inversely proportional to the length of the tube l , and also proportional to a constant k which is equal to 0.18 for oxygen and standard temperature at the selected units.

If we know the volume of inflow S per second and if we measure a and l , the differential pressure $p-p_1$ necessary for diffusion can be calculated from the formula. Such calculations were carried out by me for several tracheal systems on the basis of the necessary measurements, in order to determine to what extent diffusion will be adequate to supply oxygen or not.

Similar considerations are applicable to the discharge of carbon dioxide. A specific investigation for the problem of this gas is superfluous. The circumstances are so similar that it follows necessarily that the discharge of carbon dioxide by diffusion is possible if we have demonstrated the possibility of supplying oxygen in this manner.

The necessary measurements are as follows:

1. The oxygen consumption of an insect (S) can be easily determined by my microrespirometric method (Note: Krogh in Abderhalden's Handb., Vol 8). In many cases, we can restrict ourselves to an approximate estimate on the basis of the gaseous metabolism of other forms.

2. The determination of the tracheal dimensions are more difficult and cannot be ascertained absolutely. We need to determine the average length (l) of all tracheal branches calculated beginning with the spiracles. These lengths are very different and the very last terminations or ramifications can be neither perceived nor measured. We also need to know the total cross section of all tracheae (a) at different distances from the spiracles. This task can also obviously be solved only approximately.

The first measurements were carried out on specimens (preserved in ethyl alcohol) of one of the southern european myriapoda (Scutigera) in which the tracheal system has a particularly simple structure. The insect is provided with seven non-paired stigmata in the dorsal center line. Each stigma leads to an air cavity in which terminate according to Haase (Note: Haase furnished very good and exact illustrations of the tracheal lungs. Unfortunately, however, indications of dimensions are totally absent as is often the case in zoological publications so that the illustrations cannot be used for measurements), about 600 relatively short tracheae which ramify dichotomously several times. I measured the average length of the tracheae as 0.55 (5.5×10^{-2} cm) and the average diameter of the individual tracheal trunks as 0.015 mm. The cross sectional surface then is 1.7×10^{-6} cm². I proved to my satisfaction that the sum of the cross sections of the branchelets is approximately equal to that of the trunk. The probable oxygen consumption can be estimated as approximately 350 cm³ per kilogram per hour which gives us a consumption of 3×10^{-3} cm³ per animal and per second if the weight is assumed as 0.3 g. We then have

$$3 \times 10^{-3} = 0.18 (p-p_1) \frac{7 \times 600 \times 1.7 \times 10^{-6}}{5.5 \times 10^{-2}}$$

and consequently $p-p_1 = 0.0013$ or 0.13 per cent of one atmosphere.

The calculation shows that an oxygen percentage of 20.8 in the inner endings of the tracheal tubes in comparison to 20.93 per cent in the exterior atmosphere will permit an oxygen diffusion through the trachea adequate for the normal requirements of the insect. Respiratory motions are therefore entirely superfluous.

Subsequently a similar calculation was carried out for mealworms (larvae of *Tenebrio*). Gaseous metabolism for them has been determined several times and may be assumed for an animal with a weight of 0.3 grams as 3×10^{-5} cm³ per second. Simple dissection shows that the tracheal system consists principally of small groups of, on the average, four tracheal tubes starting from the stigmata. The total number of primary tracheae is about 80 and the diameter varies between 50 and 70 μ (with a mean of 60 μ). Ramification is very voluminous but could not be followed in detail. I arbitrarily assumed that the total cross section does not change during ramification and also estimated the average length as 3 mm. I therefore found $3 \times 10^{-4} = 0.19(p-p_1) \frac{80 \times \pi \times 30^2 \times 10^{-4}}{0.3}$ and consequently $p-p_1 = 0.022$ or 2.2 per cent of one atmosphere. Consequently, diffusion is here also entirely adequate for the renewal of the air in the tracheae and respiratory motions are superfluous.

It is evident that, in the cases cited and especially for *Tenebrio*, the numerical results can be claimed to have only a very limited accuracy. I therefore attempted to find a method which would permit more accurate measurements. The objective was an easily understandable preparation of the entire tracheal system, combined with a type of stain which would clearly demonstrate even the thinnest ramifications of the tracheae and permit measurement of their lumina. I obtained very good preparations by injection of the entire tracheal system with a coagulating fat stained as intensively as possible by alkanet or "Sudan" and subsequent decomposition of the tissues through peptohydrochloric acid. The details of methodology have been described elsewhere (Note: Krogh, Injection preparation of the tracheal system of insects, Vid. Medd. Dansk naturh., Forening 68, 1917). Fig. 2 shows such a preparation of the larvae of *Cossus*. [Fig. 2 not reproducible.]

I prepared a number of such preparations and utilized some of them for measurement of tracheae. An examination of the preparation shown will indicate that complete measurement of all branches of the trachea would require an extremely large expenditure of effort. I therefore simplified the task. I initially determined by inspection of the preparation to be measured that the tracheal ramifications starting from the various spiracles were not essentially different from each other and that therefore some of them could be selected as typical for measurement. From these, the tracheal trunks were then removed individually by means of a small pair of scissors, placed under a microscope and measured.

I began by investigating to what extent a change of the total cross section occurs through ramification. It was noted that the cross section of the trunk is usually slightly larger than the sum of the cross section of the branches. As proof, I am listing the following measurements. The figures are micrometer readings with the objective a_0 (Zeiss binocular microscope). I simply compared the square of the diameter of the trunk (D^2) with the sum of the square of the diameters of the branches (d^2).

TABLE I

| Trunk D | Branches d | D^2 | $\sum d^2$ |
|------------|--|-------|------------|
| 4. | 1.7 1.7 | 16 | 12.2 |
| 5 | 1.7 1.7 1.7 | 25 | 20 |
| 6 | 1.7 1.7 1.7 1.7 | 36 | 35 |
| 8 | 1.7 1.7 1.7 1.7 1.7 | 64 | 68 |
| 11 | 1.7 1.7 1.7 1.7 1.7 1.7 | 121 | 115 |
| | | 278 | 230 |

The difference between D^2 and $\sum d^2$ is relatively insignificant (7%) and we are therefore justified in measuring the cross section of each tracheal trunk at only one level. In the principal measurements and as far as possible, I measured the cross sections not at the trunks but only after the first ramification. For each tracheal trunk, there was also determined the average length of the branches calculated from the spiracle. These measurements were made by means of the objective F_{55} . The micrometer readings are one graduation = 0.082 mm for F_{55} and one graduation = 0.0465 mm for a_0 .

I made the following measurements on a larva of *Cossus ligniperda* with a live weight of 3.4 grams. The longitudinal trunks connecting the spiracles are not included in calculation.

TABLE II

| Trunk No. | D | Branch | ΣD^2 | L | $\Sigma D^2 L$ |
|----------------|-----|------------------------------|--------------------|----|----------------|
| Spiracle No. 9 | | | | | |
| 1 | | 2.5 2.0 1.7 | 13 | 80 | 1040 |
| 2 | | 4 x 1.5 2.0 | 21 | 90 | 1890 |
| 3 | 4 | | 16 | 50 | 800 |
| 4 | 2.5 | | 77 | 70 | 5390 |
| 5 | | 2 x 2.0 4.0 | 28 | 80 | 2240 |
| 6 | | 4 x 2.0 1.5 1.5 1.5 | 58 | 90 | 5220 |
| | | | $\Sigma D^2 = 214$ | | 14750 |
| | | | | | L = 68 |
| Spiracle No. 7 | | | | | |
| 1 | | 4 2.5 2.0 1.7 | 57 | 90 | 5130 |
| 2 | 11 | 2 x 2.0 2.0 1.5 | 113 | 80 | 9040 |
| 3 | 7 | 5 | 49 | 60 | 2940 |
| | | | $\Sigma D^2 = 219$ | | 17110 |
| | | | | | L = 78 |
| Spiracle No. 4 | | | | | |
| 1 | | 4 x 2.0 2.0 2.0 | 36 | 90 | 3240 |
| 2 | | 2 x 2.0 2.0 2.0 | 24 | 60 | 1440 |
| 3 | 6 | 2 x 4 | 36 | 60 | 2160 |
| | | | $\Sigma D^2 = 96$ | | 6840 |
| | | | | | L = 72 |

These measurements indicate that the total cross section of the trachea from the various spiracles is approximately the same. For the first spiracle, however, the cross section is definitely larger and No. 2 and No. 3 also seem to have a slightly larger cross section than the rear one. In order not to assume the conditions of diffusion as too favorable, I therefore calculated with the number 200 (instead of 220) for all 18 spiracles and moreover calculated the average length as 90 units (instead of 73). As absolute measure, I then obtained the tracheal cross section per spiracle = 0.34 mm^2 (0.37), the total cross section of all tracheae = 6.1 mm^2 (6.7) and the average length = 7.4 mm (6.0).

For control, I cut off all of the branches of spiracles 7, 8 and 9, air-dried them and weighed them, and found the weight to be 7 mg. The fat

injected has a specific weight of about 0.9. However, if we consider that the chitinous walls are included in the weight, we may estimate the volume as about 7 mm³. Calculation of volume from the above measurements produces for the three spiracles $3 \times 0.34 \times 7.4 = 7.6 \text{ mm}^3$ ($3 \times 0.37 \times 6.0 = 6.7$). Concordance is satisfactory.

Determination of gaseous metabolism in a larva of *Cossus* with a live weight of 3.5 g and the habit of slow crawling, indicated an oxygen consumption of 320 cm³ per kg an hour or 3.4 g (about 3×10^{-4} cm³) per second and per animal and we then have:

$$p - p_1 = 0.02 \text{ (0.105) or } 2\% \text{ (1.5\%)} \quad 3 \times 10^{-4} - 0.18(p - p_1) \frac{6.1 \times 10^{-3}}{7.4 \times 10^{-1}} \left(3 \times 10^{-4} - 0.18(p - p_1) \frac{6.7 \times 10^{-3}}{6 \times 10^{-1}} \right)$$

of one atmosphere.

An entirely analogous series of measurements on a larva of *Lasiocampa* with a weight of 3.9 g showed somewhat greater variations between the individual spiracles. $\sum d^2$ varied between 209 and 252. On the average, we found $230 = 0.39 \text{ mm}^2$ for each spiracle and the average length was 6.6 mm. Calculation showed the necessary differential oxygen pressure to be 1.7% of one atmosphere.

We have to disregard in these measurements and calculations that the oxygen absorption takes place only partially and probably only to an extremely minor degree in the chitinous tracheae (Note: As determined by me, *Journal of Physiology*, Vol 52, 1919), chitin is not very permeable to oxygen. The coefficient of diffusion is only 0.013 whereas it is 0.34 for water and 0.115 for the connective tissue of vertebrates. The largest amount of absorption undoubtedly takes place in the terminal network of the tracheae mentioned above. Consequently, the oxygen has to flow a further distance into the latter. The differential pressure required for this is probably very small since the distances involved are very small and constituted by very numerous, even though very narrow, connections but cannot be calculated at all at this time. An eventual mechanical respiration could in any event not affect any renewal of the air in the tracheal terminal network and diffusion is the only possibility that can be considered.

The results of the investigations cited speak undeniably for the fact that the renewal of the air in the entire tracheal system of the forms investigated takes place only by diffusion. For further control, however, I have directly demonstrated the diffusion of the air in the tracheae directly in special experiments and found that its magnitude corresponds at least approximately to the measurements and calculations on hand in the given formula.

These experiments were carried out on larvae of *Cossus* under ether narcosis. The narcosis positively prevents any mechanical respiration. The insect becomes completely relaxed but shows an oxygen absorption of about normal magnitude, about 200 cm³ per kg an hour which in itself proves that this volume of oxygen is capable of flowing in by diffusion. Such an insect was stretched out horizontally with one row of spiracles pointing

upwards as shown in Fig. 3. Around all of the spiracles of the top row, the skin was well greased with vaseline. Over the greater number of the spiracles, small bells of collodium with a volume of 6-10 mm³ were inverted but small tubes of a length of 1 cm, also made from collodium, were placed over some of the spiracles.

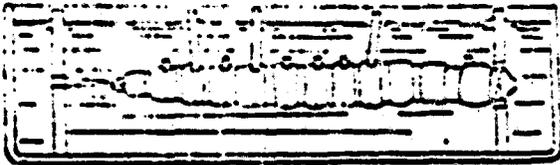


Fig. 3

The entire preparation was placed in a flat glass dish into which water was filled carefully until only the opening of the tubes remained free. Gaseous metabolism can then take place only through the spiracles equipped with the tubes and the oxygen must diffuse from these into the longitudinal tracheal trunks. Gradually an equilibrium will be established and the gas pressures in the bells must adjust in accordance with the conditions of diffusion and the oxygen consumption in the various segments. After a suitable interval, the bells were therefore removed successively and their content determined by micro-gas analysis.

We carried out several such experiments:

1. Larva of *Cossus*: weight 3.5 g; spiracle 1 open on left side, all others covered with bells. Placed under water at 1:30; analyzed at 2:40; spiracle No. 4 contained 2.4% CO₂ and 1.2% O₂.

2. Same insect: tube on spiracle 2; placed under water 4:15; analyzed 5:00; spiracle 3 contained 2.6% CO₂ and 4.8% O₂

3. Same insect: tube on spiracle 2; placed under water 3:25.

Analysis

| Time | Ambient Air | CO ₂ % | O ₂ % |
|------|-------------|-------------------|------------------|
| 4:15 | 2.4 | 2.4 | 1.2 |
| 5:00 | 2.6 | 2.6 | 4.8 |

4. Larvae of *Cossus*: weight 4.2 g; tubes on spiracles 2, 6, 8 (Fig. 3); placed under water 3:00.

Analysis

| Time | Spiracle No. | CO ₂ % | O ₂ % | O ₂ difference |
|------|--------------|-------------------|------------------|---------------------------|
| 3:00 | 2 | 2.4 | 1.2 | 1.2 |
| 3:15 | 2 | 2.6 | 4.8 | 2.2 |
| 3:30 | 2 | 2.8 | 8.4 | 5.6 |
| 3:45 | 2 | 3.0 | 12.0 | 9.0 |
| 3:00 | 6 | 2.4 | 1.2 | 1.2 |
| 3:15 | 6 | 2.6 | 4.8 | 2.2 |
| 3:30 | 6 | 2.8 | 8.4 | 5.6 |
| 3:45 | 6 | 3.0 | 12.0 | 9.0 |

The animal was injected subsequently and measurement of the longitudinal trunks showed a diameter of 0.70 mm. The lengths of the trunks from spiracle to spiracle are slightly larger than the corresponding external distances.

The first three experiments were made solely for orientation and indicate that there is undoubtedly gaseous diffusion through the longitudinal tracheal trunks which is, however, insufficient for the gaseous metabolism of the insect if it must take place over long distances (Note: We know from the investigations of G. Adler (Skand. Arch. Physiol. Vol 35, 1917) carried out in my laboratory that a differential oxygen pressure of about 6% between the atmosphere and the tissues of the larvae of *Cossus* is required for the inflow of the volume of oxygen necessary for normal gaseous metabolism. If we therefore find less than 5% oxygen in one of the balls, a lack of oxygen must have occurred in the respective segment.).

Experiment no. 4 makes it possible to carry out at least an approximate comparison between calculated and observed magnitude of diffusion. If we assume, for example, that a volume of oxygen adequate for two segments or 1/5 of the insect must pass from spiracle 6 through the trunk to spiracle 5 and if we calculate the differential oxygen pressure required, we then find $p - p_1 = 5.7\%$ if we assume the path of diffusion as 8 mm. Analysis showed 6.8%. For spiracle 1, the gaseous metabolism can be estimated as 1/8 of the entire insect and the path of diffusion as 20 mm so that we then find that $p - p_1 = 8.7\%$ whereas analysis produced 9.4%. Concordance is satisfactory and it is therefore demonstrated also experimentally that gaseous diffusion alone is sufficient to cover the oxygen requirements of the larvae.

I then attempted to investigate in detail the question whether respiratory movement or other mass movements of the tracheal air actually do occur in the larvae of *Cossus*. After some futile experiments, investigation could be carried out as follows. The rear end of a larva of *Cossus* (2.6 g) was fastened to a small fork by means of a small, short wire. The environment of spiracle 9 at the third to the last ring was well greased with vaseline and a small glass tube was pressed against this spiracle by means of a clamp. As shown in Fig. 4, this glass tube is connected by a

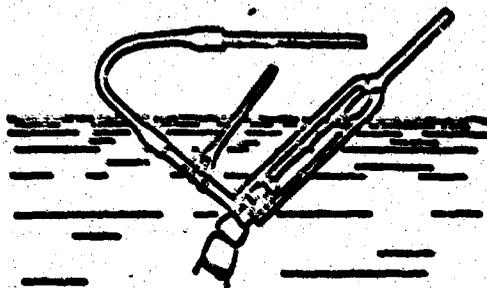


Fig. 4

rubber tube with a horizontal tube containing a drop of petroleum as volume index. The diameter of this tube was 1.7 mm and the volume of 1 mm was therefore 2.25 μm^3 . When the entire arrangement was submerged below water, all spiracles were closed off by the water and any eventual respiratory motions would therefore have been manifested by corresponding motions of the drop of oil. As long as the insect remained quiet, no motion of the drop was observed and we could only

see a constant decrease of the occluded volume of air which was due obviously to the oxygen consumption of the insect. Through general movements of the body, a minor ventilation of the tracheal system might have been produced. The latter generally amounted to only 2 mm³. With very energetic movements of the body, somewhat greater ventilation was produced. For example, I recorded at one time during a period of about 3 minutes approximately 10 "respirations" which varied between 5 and 9 mm³. As explained above, such movements are of no significance for the renewal of the air in the more delicate branchlets of the tracheae. The entire tracheal volume cannot be smaller than 50 mm³ according to the measurements.

INVESTIGATIONS ON NYMPHS OF AESCHNA

According to the results of the investigations of open tracheal systems, it is probable apparently that the transport of gas from the tracheal gills to the other parts of the body takes place by diffusion also in closed systems which was confirmed through the investigations on the nymphs of Aeschna. With a nymph of a weight of about 1 g, I carried out measurements of the tracheae. Two longitudinal main tracheae originate from each 11 + 11 dichotomously ramifying root tracheae which start from the tracheal gills. The total length of the root trachea is about 5 mm and the diameter of each main root 0.165 mm. The total cross section for each main trachea is therefore $22 \times 0.165^2 \frac{11}{4} = 0.46 \text{ mm}^3$. The main tracheae themselves have an elliptical cross section with diameters of 0.66 and 0.98 mm. The cross section therefore is 0.50 mm². The length is 40 mm. Two smaller longitudinal trunks (diameter 0.5 mm, cross sectional surface 0.2 mm²) supply the intestine. If we assume the gaseous metabolism of the insect at 300 cm³ O₂ per kg an hour and further assume that 1/3 of the oxygen must flow from the rear to the front end by way of the 40 mm long main tracheae, we then find the best required differential oxygen pressure $p-p_1 = 6.2\%$ of one atmosphere.

Calculation shows that gaseous diffusion through the tracheal system is adequate for the supply of oxygen of the tissues. However, the necessary pressure differential is so great that we may expect an appreciable increase of the metabolism to produce lack of oxygen in some tissues and it becomes evident that a relatively minor drop of the oxygen pressure in the surrounding water must lead to lack of oxygen. According to the experiments of Wallengren (Lunds Universitets Arsskrift N.F. Afd. 2, 10), emergency surface respiration starts in the larvae of Aeschna when the oxygen pressure of the water drops to 7-8%.

I have made an attempt to control the calculated differential oxygen pressure between the front end and rear end of the main tracheae by experimentation. The experiment was carried out by suddenly and as rapidly as possible pinching a nymph of Aeschna tightly in the middle by means of a clip. The front end and the rear end of the trunk were then cut off and the air in the two main tracheae of the two parts was simultaneously transferred into two instruments for micro-analysis and analysed with the

following results:

| | | | | | | |
|---------------|-----------------|------|----------------|------|------------|------|
| Front end.... | CO ₂ | 1.1% | O ₂ | 3.2% | Difference | 3.6% |
| Rear end..... | " | 1.3% | " | 5.8% | | |

which agrees well with the calculation made for the two ends of the main tracheae.

Through special experiments, I have proved to myself that mechanical transport of gas does not occur in the closed tracheal system of *Aeschna*. Since valve-like arrangements are absent, ventilation could be produced only by compressing certain parts of the tracheal system and distributing their content to the remainder of the tracheae (Note: We know that anatomically demonstrable and easily compressible parts do not exist and ventilation of this type would eventually remain very incomplete). Such compression would be manifested in an increase of pressure and a decrease of volume of the entire system. If we enclose an insect in a closed container of water as shown in Fig. 5, all variations of volume in the insect should be manifested



Fig. 5

through corresponding displacements of the water meniscus. Such displacement actually does occur. It is synchronous with the respiratory motions ventilating the terminal intestine and tracheal gills and each expira-

tion causes a decrease of volume of the tracheal content normally by 0.1 mm³ in insects with a weight of 0.8 - 1 g. With very strong expirations, we observe compression up to 0.25 mm³. The expirations are produced through compression by the entire abdomen and must produce a uniform increase of pressure in the entire body. This increase of pressure results in a minor compression of all tracheae and is consequently of no significance for the transport of gas within the system. Even if the compressions were sharply localized, they could have no significance for ventilation of the tracheal content because of their minor character.

If we examine in detail the circumstances of respiration in closed systems equipped with tracheal gills, we come to the conclusion that this function necessarily requires the tracheae to be equipped with rigid, non-elastic walls capable of sustaining high pressure without collapse. The tracheal system maintains an intensive exchange of gaseous diffusion with the surrounding water through the tracheal gills. If the tracheal walls were elastic, then the tracheal air would be subject to the pressure determined by the atmospheric pressure and the column of water above the insect, at a depth of 1 m, this would be, for example, 1.1 atmosphere. The total pressure of the gas dissolved in the water is only exceptionally (especially with abundant plant assimilation) higher than one atmosphere, but often appreciably lower because of oxygen consumption. Under such circumstances, as I pointed out repeatedly for analogous cases (Krogh, Skand. Arch. Physiol. Vol 20, 1908) and is also stressed by Winterstein (Handb. d. vergl. Physiol. Vol I, 2, p. 17), there would take place a continuous decrease of the gas volume in the tracheae until they would finally entirely collapse due to

the vacuum formed. Only if the pressure can be supported by the tracheal walls so that the occluded mass of air can be diluted to such an extent that an equilibrium of diffusion with the gases dissolved in the water becomes established, can the tracheae continuously serve respiratory purposes.

Example: We may assume that complete equilibrium of diffusion becomes established for the nitrogen which does not take part in the metabolic processes. If therefore the nitrogen percentage in the tracheal air is 90% and the pressure of the nitrogen dissolved in water 79% of one atmosphere, the total pressure in the trachea can be determined by the following simple calculation: $90x = 79$, $x = 0.88$ atmosphere. If the insect is at a depth of 1 meter, then the hydrostatic pressure is 1.1 atmosphere and the pressure supported by the tracheal walls is $1.1 - 0.88 = 0.22$ atmosphere. Obviously, the tracheae are somewhat compressed by this pressure but should not collapse.

I have attempted to expose a nymph of *Aeschna* to varying conditions of gaseous diffusion and have measured the corresponding variations of volume of the tracheal system. In the instrumentation shown in Fig. 5, I enclosed a nymph of *Aeschna* in water saturated with atmospheric air and then replaced the water as rapidly as possible by water with a total pressure of dissolved gasses of only 0.5 atmosphere. Flushing with four volumes of water was terminated after two minutes and the meniscus was adjusted in the field of the microscope equipped with an ocular micrometer. I then observed during the subsequent minutes the displacement of the meniscus. Each graduation of the micrometer corresponded to a variation of volume of 0.24 mm³. In one experiment, for example, we obtained the following readings:

| Interval After Start of Changing Water, in Minutes | Readings | Variation in Volume, in mm ³ |
|--|----------|---|
| 2 | 80 | 1.2 |
| 3 | 75 | 4.56 |
| 3 | 30 | |
| 7 | 40 | 0.24 |
| 14 | 40 | 0.00 |
| | | Total: 24 |

Several experiments of a similar type produced very similar values and indicate that a) equilibrium of diffusion becomes established in a few minutes between tracheae and water as is to be expected in consideration of the enormous surface of the tracheal gills and that b) a pressure differential of about 0.5 atmosphere produces only a very minor compression (a maximum of 4-5 mm³ if we take into consideration the variation of volume during the first few minutes which cannot be measured) of the tracheal system.

The experiments with *Aeschna* therefore all show that mechanical ventilation does not take place or is possible within the tracheae and that the transport of oxygen takes place exclusively by gaseous diffusion.

THE SIGNIFICANCE OF THE RESULTS

There can be scarcely any doubt that the theory of tracheal respiration exposed here is applicable to all tracheata. For a large number of forms, gaseous diffusion is apparently the only method of renewal of the tracheal content which may be contemplated. For other forms, gaseous diffusion is combined with mechanical ventilation of certain tracheae or tracheal sections. As is discussed in greater detail in a subsequent communication, the renewal of air in the fine and very fine branchlets of the trachea takes place exclusively by diffusion whereas mechanical respiration is restricted to larger tracheal trunks or special "air sacs."

Insects depending exclusively on diffusion are mainly the Arachnides, Myriapoda, most of the insect larvae and probably all pupae. As far as the imagoes are concerned, it is probable but not yet confirmed by investigation that respiratory motions are absent in many small forms (Note: the comprehension of the system of air renewal is confronted by particular difficulties in the very long legs of some forms. Hansen (Entomol. Med., Vol 4, 1895) demonstrated that the extremely long legs of phalangides are equipped with two stigmata along the tibia. In grasshoppers, I demonstrated (Skand. Arch. Physiol., Vol 29, 1913) that a rapid and extensive renewal of the air in the lung and legs is possible. Whether mechanical respiration takes place here remains an open question for the time being.).

It can be easily demonstrated that the method of respiration through gaseous diffusion in a tracheal system distributed over the entire body is closely related to the size of the insects and represents only for small forms an ideal or possible respiratory mechanism. If we based ourselves, for example, on one of the larvae investigated and examined the conditions which would prevail if the insect in its linear dimensions was ten times as large (600 instead of 60 mm. long), the insect would then also have to have tracheae 10 times longer and, with a corresponding tenfold enlargement of the diameter of the main trunks and the number of the fine branchlets, the total cross section of all tracheae would be enlarged 100 times to 6.1 cm².

Since diffusion increases proportional to the cross-section and is inversely proportional to the average length, it could be increased only 10 times, i.e. proportional to the linear dimension. However, the weight of the insect would increase 1000 times (proportional to the third power of the linear dimensions) or to 3.4 kg whereas the gaseous metabolism would perhaps have to increase not 1000 times but certainly much more than 100 times, let us say 300 times. For such an insect, gas diffusion would be completely inadequate. In order to produce the same result as the tracheal system of the small insects, the total cross-section of all tracheae would then have to be enlarged not 100 times but about 3000 times (to 180 cm²) which is anatomically scarcely possible. The entire tracheal volume would then be increased from about 1.6% of the volume of the insect to about 50%.

These considerations and calculations indicate both that respiration by gaseous diffusion in the tracheae is possible only in relatively small forms such as are represented by the now living tracheata without exception (Note: Among fossil insects, there are found some forms far surpassing the now-living in size. The largest were, as far as I know, the libellule-like meganeurid of the carbon period. These forms had a length of about 30 cm and were about 3 cm wide across the thorax so that they can only be considered as rather small animals in relation to other classes of animals. Animals of this size may still have been able to breathe through tracheae, provided that the larger trunks were mechanically ventilated and the gaseous metabolism not too large. The larvae and nymphae of such insects cannot, however, have been able to breathe, like the present Aeschnidae, through terminal tracheal gills in an entirely closed tracheal system. In order to transport the required oxygen quantity by diffusion toward the head, the tracheae would then have to be wider than their bodily cross-section. I believe it to be probable that they had an open tracheal system and mechanical respiration) as well as that the conditions for gaseous diffusion become still more favorable when the dimensions are even smaller than in the cases investigated. Larvae up to a weight of 5 g can, as we have shown, rely on diffusion because their metabolism is not very intensive. In fully developed insects, oxygen absorption up to 20,000 cm³ per kg per hour has been observed. For such animals, diffusion alone is not sufficient even if they only weigh 0.1 g (e.g., bees). However, the largest number of insects are smaller and diffusion is then adequate, for all circumstances, without any mechanical ventilation of the tracheal system.

CONCLUSION

→ A formula is postulated for calculating, from the total cross-section and the average length of the tracheae of an insect, the differential pressure which is just sufficient to cover oxygen consumption by gaseous diffusion.

Measurements of the tracheal systems of some large insect larvae show a differential pressure of about 2% and diffusion therefore is entirely sufficient to explain the transport of gas in the tracheae.

Gas diffusion in the longitudinal tracheal trunks has been directly demonstrated in large larvae of *Cossus* and was measured; the absence of actual respiratory motion was also demonstrated.

It was demonstrated, by means of larvae of *Aeschna*, that the transport of gas is also exclusively supplied by diffusion alone in closed systems equipped with tracheal gills. In such forms, the tracheae must be capable of supporting high pressure without collapse in order to fulfill their function.

The general significance of gaseous diffusion or tracheal respiration is discussed and the close relations between bodily size and efficiency

of the respiratory mechanism by means of tracheae are demonstrated. For the smallest forms, diffusion is always adequate. For somewhat larger forms, it must be combined with mechanical respiration if the gaseous metabolism is intensive and even then there are rather narrow limits to possible size.

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