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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

ON THE PHYSIOLOGY OF RESPIRATION IN CULEX
(ZUR PHYSIOLOGIE DER ATMUNG BY CULEX)

[Following is a translation of an article by Dr. Edward Babak, Prague, in the German-language publication Internat. Rev. Gesamt. Hydrobiol. u. Hydrogr., Vol V, No 1, 1912, pp. 81-90.]

From Observations and Experiments by J. Hepner
Under My Direction

Occupied with comparative investigations of the respiratory mechanism among animals and having recently summarized in particular the findings on tracheal ventilation (the respective communication will be published in the near future in detail in Wintersteins Handbook of Comparative Physiology), I have given detailed attention also to the three ontogenetic stages of culex among the tracheata. I was concerned with direct observation on this suitable material on the behavior of the respiratory motions in the successive main phases of development as well as with the conditions of ventilation of the tracheal trunks in the transparent larvae of culex. So far, only very inadequate knowledge has been obtained on these subjects so that we are justified in saying that the physiology of the trachea is still in its very early beginnings. Since the dipteran larvae belong among the ecologically most interesting and, from a practical viewpoint, also most remarkable aquatic insects, this contribution may deserve some attention.

The three ontogenetic stages of culex differ greatly in regard to physiological viewpoints. The metabolism of the larva is extensively oriented to purposes of structuration so that the materials for the structure of the future fly are procured in a relatively short time; in addition to this extensive progress of assimilation, there also take place in the body of the larva extensive dissimilatory processes because a great deal of muscular activity is necessary for the procurement of nutrition and atmospheric oxygen. The pupa represents a quickly passing stage in which a quick transformation of the body is carried out. The dissimilatory processes seem to be more restricted than in the larva, to judge from the manifestations of life, although intense locomotor reactions are produced by

interference with the insect on the surface of the water. The fly is distinguished by very pronounced dissimilatory processes. Flight as well as procurement of nutrition and sexual function probably place high demands on metabolism. It would be of considerable interest to investigate these three stages of development in regard to their oxygen requirements, which is what we intend to do sometime in the future.

However, the reaction of the larva, pupa and fly to oxygen deficiency is also noteworthy, even though we cannot yet arrive at any definite opinion on the intensity of the oxygen requirements and can inform ourselves specifically only on the sensitivity of the central nervous system to oxygen deficiency. The experiments carried out at the same temperatures actually did show us that extraordinary differences do occur here. We hermetically enclosed larvae and pupae in distilled water or placed them in a small amount of liquid in an aerated chamber under the microscope and then conducted hydrogen through the chamber. The flies were placed at a certain moment in a flow of hydrogen which displaced the small amount of air in the vessel in which they were contained. In the hydrogen-filled chamber, paralysis due to oxygen deficiency occurred, on the general average, after 95 minutes with the larvae and after 55 minutes with the pupae. In contrast, recently hatched imagoes displayed paralysis due to oxygen deficiency within an interval of less than 100 seconds. After enclosing the larvae and pupae in thoroughly boiled water, entirely similar findings were obtained as with hydrogen and with the same difference in time between larvae and pupae. The former become paralysed within about 90 minutes and the latter within about 50 minutes. If customarily aerated water is utilized for enclosure, we then obtain an appreciable lengthening of the interval of time until the occurrence of paralysis. The larvae subsist for about 4 hours and the pupae for about 2 hours (cf. also skin respiration). Finally, as will be seen from the following, the larvae and pupae can also be exposed to asphyxiation by (at least partially) removing the ventilation syphons and/or the prothoracic stigmata. A similar interval elapses until the occurrence of asphyxia as in the experiments with boiled water or hydrogen already mentioned.

We may conclude from this that those manifestations of life in the central nervous system which require continuous oxygen supply, stand in the forefront for the imaginal stages whereas the anoxybiotic processes have great significance for the pupae and even more for the larvae. It may be probable that we may extend this concept to the entire process of life in the early forms of the three developmental stages. We may refer in this respect to similar findings from the investigations conducted by Amerling (On the Resistance to Oxygen Deficiency during the Ontogeny of the Frog, Pflug. Archiv. 1908, Vol. 71, p. 363), under our direction, on the successive developmental stages of the frog where the early embryonic forms are shown to be highly independent from the oxygen supply; subsequently, with increasing growth and development, the sensitivity of the central nervous system to oxygen deficiency gradually increases. (Similar findings seem to exist also in regard to the embryonic stages of birds and mammals and even newborn rabbits, mice, etc. still show a high degree of resistance to asphyxiation.)

A link between these findings of the experiments for asphyxiation of the three ontogenetic main stages of culex and the particularities of the metabolism of the latter would be possible only after further investigation since it would be necessary, at least, to determine the relative oxygen consumption. However, one fact is already evident: the relative sensitivity of the central nervous system to oxygen deficiency is closely related to the development of the respiratory motions. Neither in the larvae nor in the pupae can we observe any indications of specific respiratory motion or even of rhythmic ventilation, not even in the prolonged experiments for asphyxiation. We find here also an analogy to the circumstances which were confirmed by us (Note: Babak and Kuhnova, On the Respiratory Rhythm and the Ontogeny of the Respiratory Motions in the Urodela, Pflug. Archiv. 1909, Vol. 80, p.444) in the amphibia. Young larvae of amblystoma do not manifest any respiratory motion but, in sequence with the growth of the body with which the growth of the body surface does not keep step, the latter begins at the same time as the oxygen requirements increase, in order to facilitate gaseous metabolism.

In the imago of culex, the respiratory oscillations of the abdomen are primarily noticable in the tergites which fall and rise with expiration and inspiration. As a rule, we find here a periodic increase and subsequent lesser frequency of the respirations. If the insect is observed while it recovers from the paralysis due to oxygen deficiency, we first note a persistent arrest of respiration followed subsequently by very slight and infrequent oscillations and/or also small groups of heavy respirations, again followed by intervals. Gradually the groups become more extensive, but heavy respirations are more infrequent and smaller breaths prevail. The pauses between the groups become shorter and finally join into a continuous, uninterrupted and slightly oscillating rhythm built, in regard to amplitude and frequency, of the periodic ("interrupted") respiration.

In the area of the first to the fourth abdominal segment, it is possible to observe, a few seconds after the start of exposure to hydrogen, a rapidly increasing and very perceptible swelling or insufflation which appreciably deforms the body. The swelling begins to decrease a few seconds after removal of the hydrogen and soon disappears completely. We intend to carry out further investigations on the significance of this particular manifestation.

Consequently, specific respiratory movement is completely absent in tracheal ventilation of the larva and the pupa. Very probably, this extensive auxilliary of respiratory activity does not develop when oxygen requirements are low and we do know that the tracheal system often secondarily largely atrophies (in small forms, etc.). On the basis of the present findings on the respiratory motion which ventilates the tracheae of the insects, we can state that the respiratory movements displace the gas content of the large tracheal trunks and partially renew it from the exterior through the stigmata; for the essential significance of the renewal of air, we can point here from the comparison to the fact that the insects possessing

closed tracheal systems do not have any corresponding respiratory motion -- which could here only serve the intermixing of the air. However, in the closed tracheal systems, we should not overlook the possibility that their gas content consists primarily of oxygen (obtained through the secretory activity of the skin or the tracheal gills and/or capillaries) the displacement of which is unnecessary. Moreover, we have found that the respiratory motions of Dytiscus and those of Locusta (according to Trevianus, as we have discovered subsequently) continue also under water -- with Dytiscus after removal of the dytra and wings. We are therefore of the opinion that the respiratory motions in the open tracheal system serve not only for the renewal of air but also for its displacement in the larger tracheae. The further transport of oxygen into the tracheal capillaries of the tissues is probably produced by "aspiration" of the oxygen and the consequent rapid diffusion (the participation of the closure mechanism -- the stigmata -- in such transport appears to us so far completely hypothetical on the basis of our review of the literature).

What can then be, in the larva and pupa of culex, the forces which serve the intermixing of the gas content in the large tracheal trunks (even we admit that the lower oxygen requirements of these insects do not require any extensive auxilliaries for facilitating gaseous metabolism)? They are, on the one hand, the powerful vibrations of the abdomen which produce locomotion and undoubtedly exert strong expansion and pressure on the tracheal trunks. We may perhaps also have to assign a respiratory significance to the pulsations of the dorsal vessel as earlier researchers have occasionally done; actually we see that the two longitudinal tracheal trunks of the larva alternately approach and become displaced from each other (in particular in the distal abdominal segments) in which the laterally outgoing branches are often appreciably stretched. The striking tachycardia observed during the experimental asphyxiation (cf. below) might also be invoked in confirmation of this concept of the respiratory significance of cardiac activity.

Ventilation in the larva takes place through the respiratory appendix of the next to the last abdominal segment where the stigmata of the two longitudinal tracheal trunks end in a uniform terminal chamber. At the end of the "respiratory syphon" (cf. in detail: Raschke, On the Anatomy and Histology of the Larvae of Culex Nemorosus, Zool. Anz. 1887, Vol. 10, p.18; The Larva of Culex Nemorosus -- A Contribution to the Knowledge of Insect Anatomy and Histology, Arch. F. Naturgesch, 1887, Vol. 53, p.133), there is located the one external closure mechanism and, at the terminations of the stigmatal tracheal section in the terminal chamber mentioned, the other internal closure mechanism. The pupa ventilates by way of the two earlike "prothoracic cornua;" according to Miall (The Natural History of Aquatic Insects, London 1903, p.104), the latter are protected in the interior against the entry of water by a layer of bristles, but we believe that the narrowness of the tube formations itself is completely adequate for this. In both stages, we were able to confirm the great sensitivity of these ventilation devices to the normal air atmosphere. When enclosed hermetically under water, the respiratory cornua, so-to-speak, feel along the glass cover

and are able to locate tiny air bubbles and then converge on such air bubbles with the extended terminals and even draw the bubble with them down into the water. Air bubbles are also sought and located at the bottom of the vessel and grasped by the respiratory cornua (even in a side and horizontal position of the body). The end of the larval respiratory tube with its valvular devices here unsuccessfully pants for air. If a hydrogen atmosphere is provided above the water, we then see that the syphon terminal is sometimes closed on the latter or else opens and closes quickly, but it is rather doubtful, in view of the restlessness of the insect, whether the larva ventilates its tracheal system with hydrogen. Very probably, the foreign gas irritates the syphon receptors and the inner closure mechanism remains shut. As we will describe in detail below, the gas content of the tracheal trunks is soon "emptied" and, if larvae with the principal tracheae emptied of gas in various ways (cf. below) are placed in water with a hydrogen atmosphere, the trachea remain definitely empty. The respiratory cornua of the pupa remaining at the hydrogen atmosphere then incline -- evidently irritated -- back over the dorsal surface of the body.

Both the terminals of the respiratory cornua as well as of the respiratory tube prove themselves essential devices for the normal ventilation of the tracheal systems. When these segments are removed, the pupa and the larva remain instinctively at the surface of the water in contact with air but ventilation is no longer carried out. The insects become asphyxiated within the same interval as when they are hermetically sealed in water or when the stigmata tubes are ligated; the tracheal system is found empty within a few minutes (cf. below). We are unable to say on what this essential significance of the external tracheal terminals is based (in the larva where the closure mechanism during asphyxiation often effects rapid rhythmical movements, we might conceivably think of a sort of pumping motion. This idea is, however, improbable and can moreover be refuted by direct observation. If the larva is made to "asphyxiate slowly" i.e. if the effect of oxygen deficiency is restricted to just "emptying" the longitudinal tracheal trunks, the larva is able to recuperate and we then see it continuously suspended at the surface of the water but the filling of the tracheal system takes place extremely slowly and it is not possible to observe any kind of "pumping action"). Consequently, we consider the rhythmic oscillations of the closure mechanism of the respiratory tube which now and then are shown under contact with the hydrogen atmosphere, only as the effect of irritation.

The asphyxiation experiments discussed above indicate that appreciably different intervals elapse before paralysis, depending on the oxygen content of the water in which the larvae and pupae were hermetically enclosed. According to Raschke, such culex larvae survive for ten hours; according to Rysell (Contributions to the Biology of Mosquitoes, Arch. f. Ship and Tropical Hygiene, 1907, Vol. II; Ref. Neap. Jhb.), they survive for days below the ice cover. (However, below the ice; large air bubbles accumulate which enable the insects to ventilate extensively (cf. below). This is done by gill and skin respiration and, according to Raschke, also by intestinal respiration. In regard to the gills, Jaworowski states (On the Tubular Appen-

dices of the Larvae of Nematocerae, Kosmos 1885, Vol. 10, p.204) that the contraction of the internal stroma of the anal gill appendices introduces blood into the abdominal cavity which is subsequently returned to these appendices. However, not even in the most extreme asphyxiation experiments were we able to see any noticeable displacement of blood here. Even so, we believe these appendices to be also respiratory organs but to us they seem to act more as tracheal (and not as true or blood-filled) gills. If we removed these gill platelets, the insects then all died more quickly than normal insects in hermetically closed and aerated water. However, the main part of surface respiration incumbs to the general surface of the skin. We were also not able to convince ourselves in regard to the intestinal respiration postulated by Raschke (cf. below).

The extent to which the tracheal system with its ventilation is important for gaseous metabolism is illustrated by the observation that a larva or pupa hermetically enclosed in a large amount of oxygen containing water together with a tiny air bubble has lived for days in full health by deriving from this air bubble an adequate volume of oxygen. The insect can even be kept alive by a hydrogen bubble because new oxygen continually enters the bubble through diffusion from the surrounding water. The interrelation of tracheal and of skin respiration can also be demonstrated by placing the insects in water either containing much or little oxygen but with access to the air. In connection with the insignificant skin respiration in the latter case, we frequently see them go toward the surface of the water and/or rest here longer than in the former case.

In conclusion, we want to describe the manifestations during asphyxiation in greater detail and devote attention particularly to the behavior of the tracheal system in this.

We have already intimated above that asphyxiation can be produced in several ways. Either we enclose the larva and pupa in a small amount of water in a chamber ventilated by hydrogen or in boiled and eventually -- if asphyxiation is to take place more slowly -- in the usual aerated water. It is also possible to cut away the external terminations of the stigmata or to ligate the respiratory tubelets with a silk thread. We then observe high general unrest, intense locomotion and sallies toward the surface of the water; the end of the respiratory tube is brought into contact with the oral appendices ("preening"); the respiratory tubelets of the pupa are activated intensively; etc. After a few minutes, the cardiac pulse is already accelerated, e.g., from about 60 to over 200 per minute, at the same temperature. This was also observed with Nepa, Ranatra, and others (see also our experiments on fish embryos; On the Provisional Respiratory Mechanisms of Fish Embryos, Zentralbl. f. Physiol., 1911, Vol. 25). We might possibly conceive of this acceleration of the blood circulation as a regulatory device of the respiration in the surface of the skin and/or gill respiration, even though this is very slightly productive in insects. Only when asphyxiation is far advanced do we see a slowing-down of cardiac action.

There are also observed twitching movements of the larval abdomen, accompanied by spastic quivering of the rear of the posterior segment of the digestive tube and the latter occurs about 25 times per minute. In addition, there is manifested a noticeably rhythmical intestinal peristaltic of some 50 cycles per minute which is infrequent normally. We are unable to say to what extent especially the latter is a manifestation of aid to gaseous metabolism because we were not able to definitely confirm any ventilation by water of the digestive tube. There are also seen violent "rolling motions" of the so-called "salivary glands" of the thorax, and so on. The rapid onset of turbidity of the otherwise transparent body and the subsequent occurrence of uniform dark fields in the thorax (and also at the abdomen) is very noticeable.

The longitudinal tracheal trunks -- and this is the most interesting manifestation of all the symptoms of asphyxia -- are gradually emptied. In boiled water, this disappearance of the gaseous content of the large longitudinal trunks can be observed very quickly after preventing the access of air, e.g., in 10 minutes. However, even with access of air and in aerated water, this phenomenon also takes place after ligating or removing the respiratory tubelets where it is not possible to consider evacuation of the gas content by mere diffusion. It seems that here the character of the tracheal walls conditions the disappearance of the gaseous contents because we observe that the different segments of the tracheal trunks are emptied at different speeds and occasionally quite irregularly as well as now and then in an apparent relation to the segmental embedding of the longitudinal tracheal trunks which normally have a slightly wave-like trace so that there are produced rosary-like formations. Observed slightly from above, we see finally two thin threads instead of the "fully-distended" cylindrical tubes. However, in actuality, these are flat gray bands which are distinguishable by a more lateral observation.

It is then evident, from the behavior of the longitudinal trunks, that the "spiral thread or ridge" of the trachea, at least in our case, plays no role in maintaining the lumen and supporting the walls as is generally stated in literature. In the asphyxiation experiments, the spiral thread has in no case been able to prevent a collapse of the tracheal walls and the same is true when the tracheal ventilation is damaged.

The spiral thread of the culex larvae probably possesses no noticeable rigidity of any kind as may be inferred from the attempts at recuperation. If the elasticity of the thread were activated through the collapse of the tracheal walls, we should then see in the cases where the non-damaged respiratory tube of the larva is in communication with the air atmosphere (the end of the respiratory syphon always contains air because the walls of the syphon are always very rigid) or when a larva with collapsed longitudinal tracheal trunks is enclosed in oxygen-impregnated water, very quickly the filling of the tracheal trunks. However, the latter are not filled at all in the second case and only gradually in the first case. The manner in which this filling takes place also contradicts the assumed

aspirating activity of the trachea. Occasionally there is filled only one of the tracheal longitudinal trunks (and this fact also contradicts the possibility mentioned above of a pumping action of the stigmatal devices). The most striking fact, however, is that filling begins at the proximal end of the body at a time when the distal segment of the trunks located closer to the atmosphere is still empty. Through thickening in the form of a rosary, the cylindrical form of the tracheal trunks is then gradually resumed but various irregularities of the normal trace and normal thickness may persist for a longer period. However, if the asphyxiation experiment is continued beyond the critical point, there then does not occur any normal filling of the tracheal system at all. It would therefore seem as if the tracheal wall was damaged during the asphyxiation experiment so that it no longer possesses its normal characteristics and activities.

Summary:

The resistance of the central nervous system to the lack of oxygen diminishes in the ontogenetic evolution of Culex from the larva to the pupa and especially to the imago progressively. The special respiratory movements are developed in connection with this in the imago alone; it is also possible to find in the latter a typical chemical regulation of the respiratory movements (dyspnoic elevation of the rate of respiration).

The author makes many remarks on the physiology of the respiratory syphon of the larva and the respiratory trumpets of the pupa (esp. observations and experiments concerning the essential signification of the extremity of these organs), on the behavior of them in hydrogen, the respiratory function of the (tracheal) gills of the larva, the cutaneous respiration and the respiratory signification of the distal extremity of the alimentary tube.

The phenomena during the asphyxia in the body of the larva are described, especially, how the two main tracheal trunks, though there is distinctly developed a spiral thread in their wall, are flattened and "emptied," a behavior which differs greatly from the hitherto supposed merely mechanical function of the chitin wall of the air-tubes; the living wall of the air-tube is probably an active instrument in the normal ventilation of the trachea.

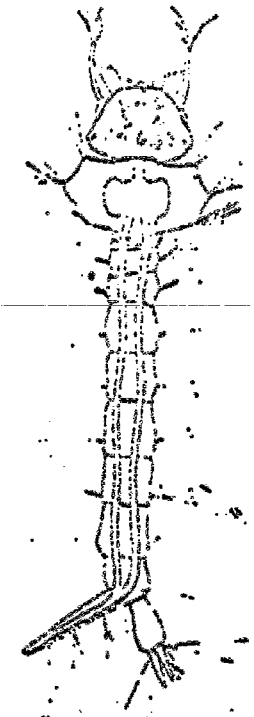

Fig. 1 - Larva of culex with fully distended principal tracheae.

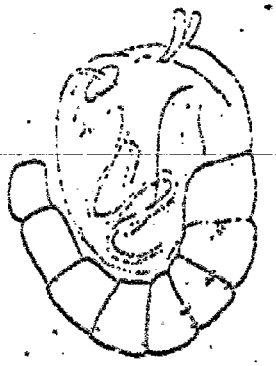

Fig. 2 - Pupa of culex.

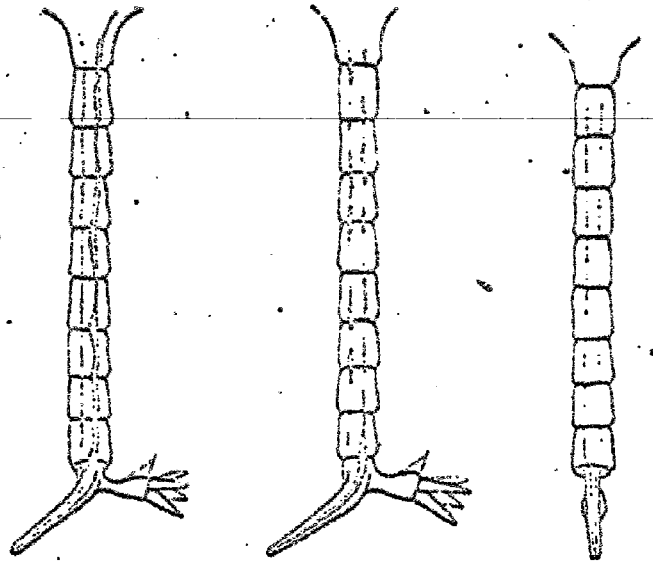

Fig. 3 - Various views of the posterior body of larvae of culex undergoing asphyxiation; the emptied principal tracheae appear as more or less uniform flat bands or threads.