THE IMPORTANCE OF THE BALANCE OF HIGH-ENERGY COMPOUNDS IN THE ACTIVITY OF THE RESPIRATORY CENTER

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The frog is an animal which stores air in its lung sacs. Pulmonary respiration in this animal is regulated by oxygen lack [13, 19] and not by accumulation of carbon dioxide; the latter is mainly eliminated via the skin [20]. When the animal is in a state of motor rest, the lung sacs are moderately filled with air, and the frog performs only oro-pharyngeal respiratory movements with the nares open and the vocal cleft closed, i. e., it ventilates the oro-pharyngeal cavity. When the frog is in anoxia, it develops a typical respiratory rhythm consisting of the periodic appearance of groups of respiratory movements, expanding and contracting the lungs. During the pauses betwen the groups of respiratory movements, the lungs remain inflated, and arterialization of the blood continues. The fact that this type of respiration is dependent upon anoxia was first established by the Czechoslovak physiologist Babak [11, 12]. The pulmonary ventilation is considerably increased by this type of respiration [5], which develops when the grass frog is placed in an environment with a low partial pressure of oxygen [11], in hydrocyanic acid poisoning [5, 18] and in certain severe circulatory disturbances (clamping of the aorta, exsanguination of the animal, administration of poisons causing slowing or arrest of the heart [11]). This type of respiration also arises after curarization of the frog: in the stage preceding paralysis of the respiratory musculature, the oro-pharyngeal rhythm is replaced by periodic respiratory movements of the lungs, inflating the lung sacs (the amplitude of these movements remains very low under these circumstances); injection of proserine into the curarized animal leads to restoration of the respiration, characterized by periodic respiratory movements [7].

Thus while the frog displays great tolerance to oxygen lack, the innervation of its pulmonary respiration is characterized by high sensitivity even to transient anoxia.

Oxygen is utilized by the body during respiration for the formation of high-energy phosphorus compounds. It is therefore possible that anoxia causes dyspnea in the frog by disturbing the positive balance in the metabolism of high-energy compounds.

We know of no such relationships in warm-blooded animals. Stimulation of respiration here arises by a reflex mechanism from the chemoreceptors of the cartoid sinus under the influence of various factors: anoxia, and poisoning both by substances disturbing the utilization of oxygen by the tissues, e. g., cyanides [16], azides [1], and by substances disturbing the phosphorylation associated with respiration, e. g., dinitrophenol [21] and methylene blue [3]. The mutual interaction of these factors, leading to disturbance of the positive balance in the metabolism of the high-energy compounds in the receptors of the carotid sinus, was examined by Belen'kii [4].

EXPERIMENTAL METHOD AND RESULTS

In this research an attempt was made to produce dyspnea in the frog (Rana temporaria) by poisoning it with α-dinitrophenol, a poison disturbing the respiration associated with phosphorylation. Observations were made both on free animals and on animals fixed to an experimental board. In the latter case we recorded the respiratory movements of the muscles of the floor of the mouth (after making a small incision in the skin the muscle fibers

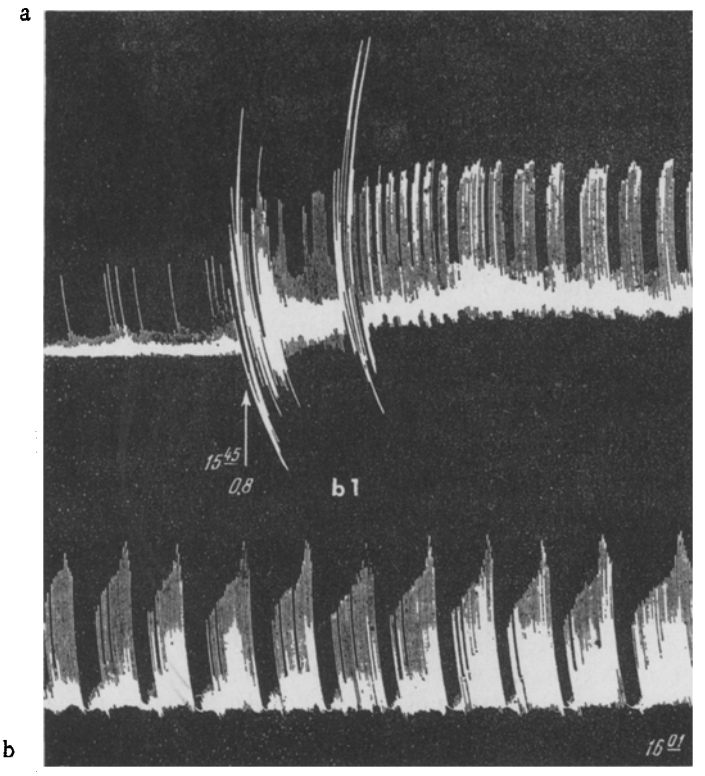

Fig. 1. Recording of the movements of the floor of the mouth of a frog (experiment No. 30, April 1958, female frog weighing 42 g). a) Oro-pharyngeal respiration, occasionally interrupted by pulmonary respiratory movements. Periodic pulmonary respiration begins 1.5 minutes after the subcutaneous injection of α-dinitrophenol (the solution was injected while the drum was rotating, the moment of injection being indicated by an arrow). In the pauses between the pulmonary groups the respiration is oro-pharyngeal, and in this case the oro-pharyngeal rhythm has a larger amplitude than normally; b) continuation of the experiment 7 minutes later. The periods of pulmonary respiration become more prolonged, and the staircase type of elevation is more marked, each pulmonary respiratory movement being stronger than that preceding it. Immediately after the last pulmonary respiration there is a short pause. The number of oro-pharylgeal respiratory movements heralding the pulmonary period becomes increasingly smaller.
Key: b1 = 0.2% DNP (~40 μg/g).

were caught by the hook of a dried burdock bract; a thread passed from this bract to the recording lever).

Experiments on 42 frogs showed that the subcutaneous injection of α-dinitrophenol, starting with a dose of 5 μg/g body weight, approximately one quarter the lethal dose causes a dyspneic reaction similar to that which arises during anoxia or hydrocyanic acid poisoning (Fig. 1).

With nonlethal doses of dinitrophenol this periodic respiration continued for many hours; the duration of the pauses gradually diminished, and between the groups of respiratory movements inflating the lungs, other respiratory movements "ventilating" the lungs were interposed, and respiration became continuously pulmonary.

During the respiratory rhythm of "ventilation" of the lungs, the pulmonary "polypnea" was more marked than during the periodic inflation of the lung sacs. The increase in pulmonary ventilation here is, however, problematical, for what took place was simply the oscillation of the same volume of air without renewal, from the

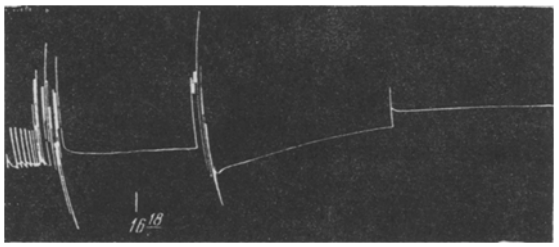

Fig. 2. Recording of the movements of the frog's mouth (continuation of experiment No. 30, recording after 15 minutes). The groups of pulmonary respiratory movements are shortened, the pauses are much longer, and before respiration ceases it becomes gasping in character.

lung sacs into the oro-pharyngeal cavity and vice versa. Babak [11] inclines to the view that in this type of respiration there is possibly some degree of renewal of the air in the lungs, because of incomplete closure of the mouth. He nevertheless considers that this variant of respiration is evidence of the considerable functional disturbance of the respiratory center, arising as a result of prolonged oxygen lack. In this way only that pulmonary polypnea which is characterized by the periodic inflation of the lung sacs may be regarded as dyspnea leading to a significant increase in pulmonary ventilation. The rhythm of "ventilation" of the lung arising in stage 2 of dinitrophenol poisoning was not observed in cyanide poisoning.

With lethal doses of dinitrophenol, instead of the onset of a rhythm of "ventilation" of the lung, there was a shortening of the groups of pulmonary respirations inflating the lungs, the pauses became lengthened (Fig. 2) and, finally, the respiratory movements ceased altogether.

The experimental findings enable two fundamental problems to be analyzed: 1) the reflex or centrogenic nature of the dyspnea caused in frogs by anoxia or poisoning by cyanide or dinitrophenol, and 2) the biochemical mechanism of development of the dyspneic reaction. The work of Karasik [5, 6] has shown that hydrocyanic acid causes a dyspneic reaction in Rana temporaria even after division of the glossopharyngeal nerve, and on this basis he denies that a reflex from the carotid bodies plays any part in its production. The reaction also took place after vagotomy, and therefore the dyspneic reaction evidently does not depend upon a reflex from the surface of the lung. A few years ago, however, after painting the carotid bodies in Rana esculenta with phenol, Smyth [22] did not observe dyspnea in the animal when it was placed in an atmosphere of nitrogen, and he concluded from his observations that the reflex regulation of respiration in warm-blooded animals and amphibians was similar.

The importance of the chemoreceptive vascular zones in the regulation of respiration in amphibians was stressed by Kravchinskii [9]. In experiments on Rana ridibunda, carried out in the climatic conditions of the south (Samarkand and Sochi), at a "high temperature and with bright illumination," he observed that vagotomy above the branching of the long laryngeal nerves, and division of these nerves or division of the aortic nerve, which he identified, led to the rapid (in the course of a few minutes) paralysis of respiration and to the subsequent areflexia of the frogs (he did not observe this phenomenon in experiments on Rana temporaria in Leningrad). Painting the trunk and bifurcation of the aorta with solutions of nicotine or potassium cyanide caused "strengthening and quickening of respiration" (in accordance with the high sensitivity of the aortic reflexogenic zone to these chemical agents Kravchinskii compares it to the aortic and carotid sinus zones in mammals).

The strengthening and quickening of respiration cannot, however, be regarded as a dyspneic reaction in the frog. This reaction, as we have pointed out above, is characterized by periodically developing groups of respiratory movements inflating the lung sacs, and although these movements become stronger, their total number is smaller than normally when frequent oro-pharyngeal respiratory movements are predominant.

Particular attention should be paid to the experiments with nicotine. In a paper by Karasik [6] an examination is made of the inaccuracies repeatedly arising in the work of several authors in their attempts to use drugs (for example lobeline), which can be used as "respiratory analeptics" in mammals, for the "stimulation" of respiration in the frog. These misconceptions have arisen as a result of the identification of physiological processes which differ very considerably in these animals, as a result of the inadequate differentiation between oro-pharyngeal and pulmonary respiratory movements, as a result of inadequate regard for the physiological role of the periodic activity of the lungs as a dyspneic reaction, and so on. According to Karasik's findings, lobeline does not cause a dyspneic reaction in the frog (Rana temporaria); it can hardly be expected, therefore, that such a reaction will arise under the influence of nicotine (provided, of course, that the slowing or arrest of the cardiac contractions and the associated oxygen lack are prevented). The same remarks apply to the experiments of Boelaert [14], using nicotine and lobeline.

Smyth's experiments were repeated by Belen'kii [2]. Initially, in agreement with Smyth, Belen'kii did not

observe dyspnea when cyanides were administered to Rana temporaria in which the carotid bodies had preliminarily been painted with phenol. As the techniques improved, however, he began to obtain positive results: dyspnea developed after both painting the carotid bodies with phenol and resecting them. Belen'kii reached the same conclusions as Karasik, and explained Smyth's negative findings by the fact that the experimental animal was in a very poor condition after undergoing the operative and chemical trauma (it was incapable of reacting with a dyspneic reaction to the injection of cyanides).

Since these different authors had carried out their experiments on different species of the genus Rana, at different seasons of the year and under different climatic conditions, and had not always taken into consideration the physiological peculiarities of the frog's respiration, the problem of the presence of vascular reflexogenic zones in this genus of frogs, taking part in the regulation of respiration and in the development of dyspnea, cannot yet be regarded as solved. At this point we must mention the work of Boelaert [14], who showed that in reptiles (Lacerta ocellata) anoxia, cyanide poisoning and also lobeline and nicotine poisoning are accompanied by the development of dyspnea even after denervation of the vascular zones homologous with the carotid sinus and the cardio-aortic zone in mammals. In Boelaert's opinion this dyspnea is centrogenic in nature.

The discussion of the second problem must begin with the recollection that until recently the prevailing opinion was of the dominant role of acid products of metabolism in the regulation of respiration (the history of this problem is described in detail in the monograph of Cordier and Heymans [15]). The importance of pH changes in the respiratory center to its excitation was defended for a long time by Winterstein, and this opinion has been held by some writers [23] until the present time, among them some who have studied the influence of α-dinitrophenol on the respiration of warm-blooded animals [24]. Subsequently Winterstein localized the development of the respiratory excitation as depending on a change of pH in the carotid sinus and not in the center (cited by Heymans [17]).

This theory, however, still fails to explain how the acid products appearing during anoxia (or the change in pH) affect the excitable structure. It has been known for the last two decades that all vital processes which have been adequately studied from the biochemical point of view are brought about by energy accumulated in high-energy compounds, and in discussing the present problem this fact must be taken into consideration. A decisive step in its development was the discovery by Lyubimova and Engel'gardt [10] that the contractile properties of myosin are indissolubly connected with its adenosinetriphosphatase capacity.

As a result of the analysis of the experimental evidence showing that poisons affecting the associated phosphorylation (dinitrophenol, methylene blue, gramicidin, monobromoacetate, etc.) cause a sharp increase in the strength and frequency of contraction of skeletal muscle caused by guanidine, one of us [8] suggested that the excitable protein of the neuromuscular synapse is itself a high-energy compound, and that excitation is characterized by the breakdown of its high-energy bonds. The same suggestion may be made with regard to the dyspneic excitation caused by anoxia or by poisoning with cyanides and dinitrophenol.

SUMMARY

Poisoning of Rana temporaria with alpha dinitrophenol provokes periodic pulmonary respiration characteristic of dyspnea in this animal. The mechanism governing the appearance of this reaction is connected with the disturbed balance of high-energy compounds. The question of its reflex or centrogenic nature is discussed.

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