Thermochimica Acta, 229 (1993) 193–204
Elsevier Science Publishers B.V., Amsterdam

Metabolic rate and tolerance of anoxia: microcalorimetric and biochemical studies on vertebrates and insects [1]

Thomas Moratzky, Gisela Burkhardt, Wieland Weyel and Gerhard Wegener *

Institut für Zoologie, Johannes Gutenberg-Universität, Saarstrasse 21, D-55099 Mainz (Germany)

(Received and accepted 17 June 1993)

Abstract

The acute effects of anoxia are dramatic in animals that have high standard metabolic rates. Mammals, for instance, suffer a loss of brain functions within seconds, whereas the behaviour of some lower vertebrates such as frogs appears little affected in the initial phase of anoxia so that their reactions to anoxia can be studied on an extended timescale. Adult insects have very high metabolic rates and during anoxia all organs rapidly cease to function. In contrast to mammals, however, which will suffer irreversible damage if anoxia is extended for more than a few minutes, many insects can survive anoxia for several hours in a state of rigor and fully recover when oxygen is readmitted.

This makes insects apt experimental animals for studying the question as to which structural, physiological and metabolic properties improve the tolerance of anoxia. We have followed the heat dissipation (as an index of metabolic rate) during anoxia and postanoxic recovery in frogs and in two species of insects, a locust and a moth. Frogs can survive 3–4 h of anoxia (at 20°C), locusts 4-5 h, whereas the moth *Manduca sexta* can fully recover from more than 20 h of strict anoxia.

In frogs heat flow was decreased to about 25% during 90 min of anoxia, but in anoxic insects heat dissipation was reduced much further, to about 5% in locust (during 4 h) and to less than 4% in *Manduca* (during 14.5 h of anoxia). When air was readmitted heat dissipation was rapidly increased to a level exceeding the normoxic control value (overshoot). Locust and moth differed strikingly with respect to heat dissipation during postanoxic recovery. Locusts dissipated a large amount of excess heat during recovery for more than 12 h, in contrast to *Manduca* where excess heat during recovery was small and the control value was reached within less than 2 h. Metabolic processes underlying the different patterns of heat dissipation during anoxia and postanoxic recovery are discussed.

* Corresponding author.

[1] Presented at the Tenth Ulm Conference, Ulm, Germany, 17–19 March 1993.

INTRODUCTION

Animals need free energy to execute functions such as reproduction, growth and behaviour. A substantial amount of energy is also required to maintain the high degree of order that is characteristic of living organisms. Hence, even if an animal is not noticeably active, metabolic processes must go on to provide the energy for maintaining body homeostasis. The amount of free energy for maintaining homeostasis is reflected in the standard metabolic rate (metabolic rate of a resting animal) which is different in different groups of animals and is affected by parameters such as body temperature and ambient temperature. Nutrients are degraded in the process of catabolism and energy released during this process is harvested by synthesis of ATP. The vast majority of animals are obligatory aerobes, i.e. they depend on oxygen for ATP synthesis (aerobiosis). If oxygen provision is halted, these animals have to fall back on anaerobic ATP production (anaerobiosis), when fat (which is a major fuel for energy metabolism in many animals) cannot be utilized for ATP production. Hence, under anaerobic conditions, an animal is restricted to carbohydrate as virtually the only substrate for ATP production, but the amount of ATP that can be generated from a given amount of carbohydrate is markedly reduced if aerobic ATP production is excluded. For instance, 1 mol of glucose can give rise to 38 mol of ATP if fully oxidized to CO_2 and H_2O but would yield only 2 mol of ATP when degraded to 2 mol lactate and protons. A simple estimate indicates the dilemma that is posed on an animal by lack of oxygen. Let us, for the sake of argument, consider two theoretical responses to anoxia and their physiological consequences. (1) If carbohydrate were to be catabolized at the same rate anaerobically as aerobically a severe shortage of ATP for the maintenance of cell functions would result. (2) If, by contrast, the rate of ATP production were to be kept at the same level during anaerobiosis, a dramatic increase in the breakdown of carbohydrate would be required with the consequence that substrate stores would rapidly be exhausted and metabolic products would be accumulated to toxic levels. Animals can tolerate anoxia for only a limited time span. After a period in which the effects of lack of oxygen are fully reversible, changes set in that damage vital organs to such a degree that they cannot be fully repaired when oxygen is readmitted. The interval between the onset of anaerobiosis and the initiation of irreversible damage is markedly different in different animals, ranging from a few minutes in warm-blooded vertebrates to many days in some lower vertebrates or invertebrates [1]. From the above discussion one would expect the time course of anoxic damage and hence the tolerance of anoxia to be related to the standard metabolic rate, with a high standard metabolic rate correlated with a low tolerance of anoxia. In the majority of animals studied so far this hypothesis has been shown to be correct, but there seem to be striking exceptions from this rule which call for a closer investigation into the relationship between

metabolic rate and anoxia tolerance. Direct calorimetry is an apt method for the study of this problem, but calorimetric measurements do not give information as to the metabolic pathways operating in anaerobiosis. Therefore, a combination of calorimetry and biochemistry appears a promising approach to the study of animal anaerobiosis.

ACUTE EFFECTS OF ANOXIA: ANIMAL BEHAVIOUR DURING ANAEROBIC EPISODES

Experimental anaerobiosis is often produced by changing air for an atmosphere of inert gases. Anaerobiosis brought about in this way, for instance by breathing nitrogen, is not painful, yet in mammals or birds the effects are dramatic. The effects are primarily due to a loss of function of the central nervous system, especially the brain. This has been shown on various mammals and also in man, e.g. in experiments in which in healthy volunteers, the blood flow to the brain was blocked by compression of the arteries supplying the brain (ischaemic anoxia). Consciousness was lost within 6–7 s, followed by anoxic convulsions some 10 s later. When circulation was reestablished after 100 s, the recovery was rapid and complete [1–5]. However from accidents and animal experiments it is known that in mammals complete recovery is possible only if anoxia does not exceed 5–8 min. Many lower vertebrates are much more tolerant of anoxia than mammals, particularly some cyprinoid fish such as the crucian carp *Carassius carassius,* and the goldfish *Carassius auratus* [6–8] or some turtles such as *Chrysemis scripta* [9, 10]. Frogs of the genus *Rana* are classical experimental animals in anaerobiosis research. They have been studied for about 200 years [11].

Frogs have a moderate tolerance of anoxia and do not show spectacular behavioural responses to anoxia. When a common frog (*Rana temporaria*) is exposed to an atmosphere of pure nitrogen (at 20°C) it will not normally make an attempt to escape but remain in its typical sitting position. After 2–3 min the ventilatory rate will transiently increase and then fall below the normoxic control value some minutes later. After about 20 min spontaneous movements will be significantly reduced and ventilation is not normally observed after 60 min. During a further hour in nitrogen frogs will be unable to maintain their characteristic body posture as righting reflexes will first be retarded and then gradually lost with the consequence that the frogs appear completely paralyzed.

The time course of responses to anoxia depends on several parameters such as season, nutritional state, general "fitness" and, most markedly, on the ambient temperature. Lowering the temperature from 20°C to 2°C would delay the onset of anoxic paralysis from about 2 h to several days [11]. This observation underlines the importance of metabolic rate for anoxic tolerance.

Adult insects have high standard metabolic rates and their energy metabolism is specialized on aerobic ATP production. It is therefore not surprising that adult insects show dramatic reactions when subjected to anoxia. The immediate reactions are similar to those in mammals. A locust, for instance, that is swept with nitrogen gas immediately gives up its resting position: escape movements are followed by hyperventilation (pumping of the abdomen), after 30 s, body coordination is lost and this is followed by total immobility after 60–75 s.

SURVIVING ANOXIA: ADAPTATION AND TOLERANCE

The atmosphere is rich in oxygen (almost 21%) whereas oxygen can be a limiting factor in aquatic habitats because the O_2 content of water is less than 1%. Some animals living in aquatic habitats or in muddy sediment have evolved adaptations to cope with hypoxia or anoxia (see Fig. 4 and later section). As a rule such animals are characterized by low mobility and relatively low standard metabolic rates.

Animals with high standard metabolic rates are unable to maintain physiological functions during anoxia. The acute reaction of an animal to anoxia is thus related to its metabolic rate. Surprisingly, this correlation is not necessarily true with respect to the ability to recover from an anoxic insult. As has already been mentioned, mammals would not normally recover from an anoxic interval of more than a few minutes. Many adult insects, however, although they lose all signs of life within 1 min of anoxia can survive this situation in a state of rigor and fully recover from many hours of strict anoxia [12, 13]. This fact is even more striking as most adult insects are unlikely to encounter lack of oxygen in their natural habitats. It is not known which properties of their structural and/or metabolic organization make insects so tolerant of anoxia. It is therefore interesting to study the effects of anoxia in insects because they have, like the most advanced vertebrates, a very active aerobic metabolism. However mammals are so rapidly damaged by anoxia that experimental anoxia cannot easily be studied in these animals. We shall, therefore, compare the effects of anoxia in insects with those in frogs. Frogs share with mammals the basic properties of vertebrate organization and metabolism but the effects of anoxia can be studied on an extended timescale in these animals.

DIRECT CALORIMETRY: METABOLIC RATES DURING ANOXIA AND POSTANOXIC RECOVERY

Anaerobic ATP production is less efficient than aerobic ATP production and leads to the accumulation of anaerobic products such as lactate and protons (see Fig. 4 and the following section). One obvious strategy for

surviving anoxia would therefore be to reduce the metabolic rate. Metabolic rates can easily be estimated from oxygen consumption. This is, of course, not feasible during hypoxia or anoxia, but heat dissipation or changes in metabolites can serve as indices of metabolic rates independent of oxygen availability. Direct calorimetry on several species of vertebrates and invertebrates has shown that heat dissipation is indeed reduced in anoxia-tolerant animals during hypoxic/anoxic intervals [1, 11, 13–15].

Heat dissipation during hypoxia/anoxia has been intensively studied in animals from aquatic habitats whereas studies on air-breathing animals are scarce. Two preconditions must be met for these experiments: (1) a calorimeter of relatively high sensitivity must be used because a marked reduction in heat flow rate can be anticipated, (2) defined compositions of the atmosphere in the calorimeter must be produced and maintained. The second requirement is not compatible with closed calorimeter cells, as a stagnant atmosphere would continuously change due to the metabolic activity of the experimental animals. We have met these requirements by using a twin-microcalorimeter (Calvet MS 80 from Setaram equipped with two 100 ml circulation cells) through which a stream of gas of defined composition can be pumped at a chosen flow rate. Effects of evaporation by the experimental animals are eliminated by moistening the gas before it is passed through the calorimeter cells [16].

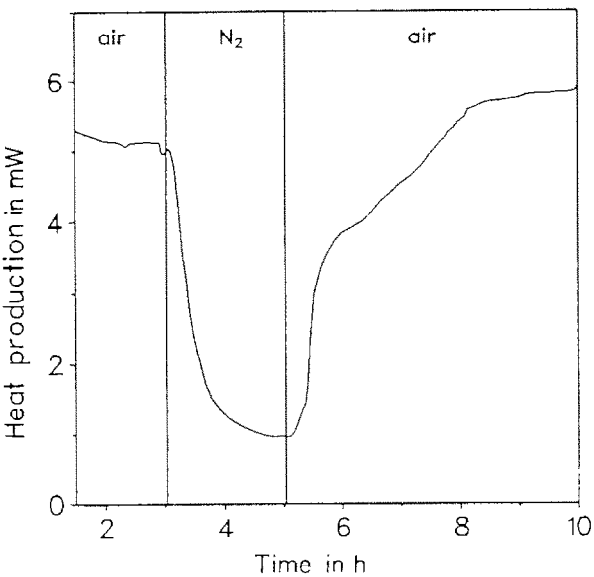


Fig. 1. Heat dissipation of a common frog (*Rana temporaria*, male, 30.6 g body mass, 20.8°C) during normoxia, hypoxia/anoxia and postanoxic recovery. The animal was placed in the experimental cell of a microcalorimeter and hypoxia/anoxia was produced by changing the gas flow (600 ± 1 ml h^{-1} per cell) from air to pure nitrogen. The frog was immobilized with curare which eliminates movements but does not provoke anaerobic metabolism in frogs because of the efficient skin respiration [16].

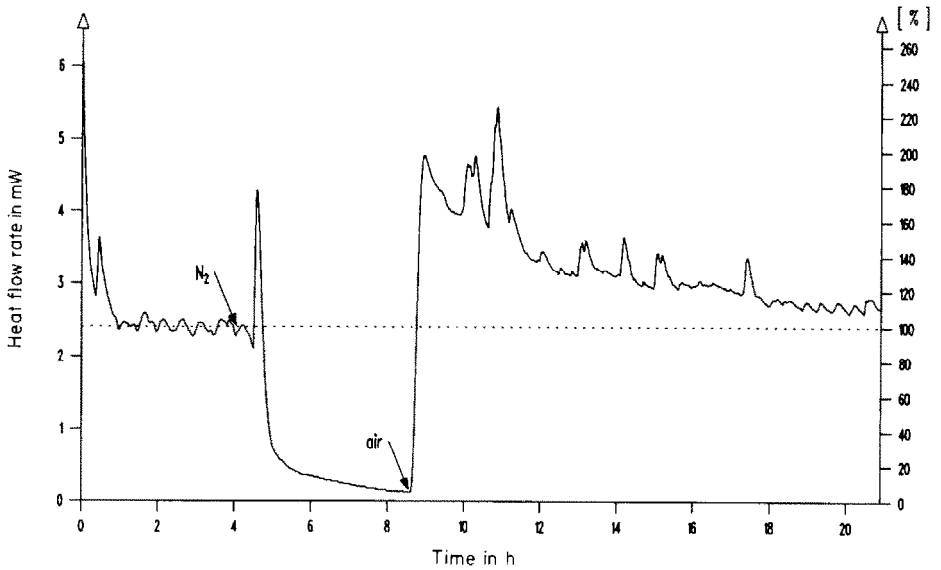


Fig. 2. Heat dissipation of an adult migratory locust (*Locusta migratoria*) during normoxia, hypoxia/anoxia and postanoxic recovery. A male locust (1.52 g body mass, 20 days of age) was kept at 20.3°C in a microcalorimeter through which a flow of gas was passed at a constant rate of 500 ± 5 ml h^{-1} (for methods, see text and ref. 16). Hypoxia/anoxia was produced by changing the gas flow from air to pure nitrogen (marked by an arrow). This provoked a transient increase in heat flow rate (due to hypoxic hyperactivity) which was followed by a precipitous decrease in heat dissipation. After 4.5 h, when the heat flow rate had reached 5.3% of the normoxic control value, the gas flow was changed to air again. Heat flow rate increased almost instantaneously, reached the control level within about 13 min and was further increased above the control level for many hours (for details, see text).

Heat dissipation of a frog during normoxia, hypoxia/anoxia and postanoxic recovery is shown in Fig. 1. Changing the gas flow from air to nitrogen brought about a precipitous decrease in heat flow rate. After about 90 min the heat flow rate had reached a steady state at about 25% of the normoxic control value. When after 2 h the gas flow was changed to air, heat dissipation was rapidly increased to a level above the control value. The time course shown in Fig. 1 and the degree of reduction in heat dissipation during anaerobiosis is typical, as similar curves have been reported for several vertebrate species. Heat flow rate was reduced to 20% of the normoxic control value in submerged toad [17], to 29% in anaerobic goldfish at 20°C [6, 7] and to 15% in turtle submerged in oxygen-free water [18]. Similar heat flow rate data have been reported for anaerobic invertebrates such as the oligochaete worm *Lumbriculus variegatus* (23% of control) [19] and the marine worm *Sipunculus nudus* (20%) [20].

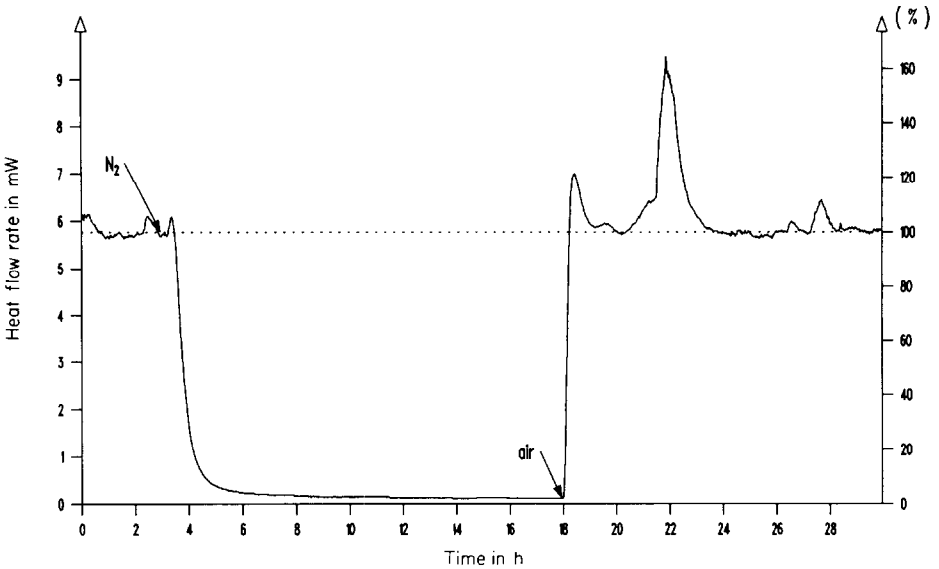


Fig. 3. Heat dissipation of an adult moth (*Manduca sexta*) during normoxia, hypoxia/anoxia and postanoxic recovery. A female moth (2.18 g body mass, 1 day old) was kept in the calorimeter as described in Fig. 2. Hypoxia/anoxia caused a marked decrease in heat dissipation. After 5.5 h of N_2 the heat flow rate had reached a constant level at 3.6% of the control value. When air was readmitted after 15 h heat dissipation increased almost instantaneously and transiently surpassed the normoxic control value (for details see text). The large peak at 22 h is probably due to wing movements.

Compared with mammals all insects can be regarded as highly tolerant of anoxia, but among insects different species may show marked differences with respect to the length of the anoxic interval they can survive [12]. At room temperature a honey-bee can survive 1 h of strict anoxia, a migratory locust 4–5 h, whereas houseflies (*Musca domestica*) and the moth *Manduca sexta* can fully recover from more than 20 h of anoxia [12, 13, 21].

We have studied heat flow in the migratory locust (*Locusta migratoria*) and the moth *Manduca sexta* in order to compare the effect of hypoxia/anoxia on two species of insects, one showing an average, and the other a high tolerance of anoxia.

The effects of hypoxia/anoxia on heat dissipation in a locust and a moth are shown in Figs. 2 and 3, respectively. Hypoxia causes a rapid decrease in heat flow rate in both insects. The minimal heat dissipation is lower than in most other animals studied so far, reaching about 5% in *Locusta* and less than 4% in *Manduca*. In both species heat dissipation was rapidly increased when oxygen was readmitted. A striking difference between the species was observed during postanoxic recovery. In *Locusta* there was a massive overshoot in heat dissipation reaching a maximum of more than 100% over the normoxic value, and even after 16 h of recovery the heat flow rate had

not returned to the control value. In *Manduca* maximum heat flow rate during recovery was only 40% above control and returned to the normoxic value within about 2 h. The difference between the two species is even more surprising as *Manduca* has a higher standard metabolic rate than *Locusta* and was exposed to anoxia more than three times as long as *Locusta.* The increased heat flow rate during recovery is a common phenomenon in animals recovering from hypoxia. It is accompanied by an overshoot in oxygen uptake. The term "oxygen debt" has been coined in this context. The excess oxygen uptake is thought to pay the oxygen debt (that the animal has incurred during anaerobiosis), and this could include refilling the body's oxygen stores (this fraction would not give rise to metabolic heat, it should be small in adult insects because these animals lack pigments for O_2 transport), resynthesis of phosphagens and nucleotides, re-establishing ion gradients and the resynthesis of substrates degraded during anaerobiosis. The overshoot in heat production is supposed to go on until normoxic homeostasis is fully reestablished. The postanoxic overshoot in heat flow rate (or the "oxygen debt") is hence an index to the degree by which the homeostasis of an animal has been affected by anoxia. The fact that in *Manduca* the postanoxic overshoot in heat flow rate is so small would thus reflect the high capacity of this species to tolerate anoxia.

It would be interesting to know the metabolic processes accounting for the decrease in heat flow rate during anoxia and to identify the mechanisms involved in the postanoxic excess in heat flow rate. Answering these questions requires additional studies because calorimetry does not give information as to the nature of the processes bringing about heat dissipation. We have therefore applied biochemical methods to study metabolic processes in frogs and insects during anoxia and postanoxic recovery.

METABOLISM DURING ANOXIA AND POSTANOXIC RECOVERY

Frogs appear hardly affected by the lack of oxygen in the initial phase of anoxia, but this impression is misleading as we have seen from the massive decrease in heat dissipation that occurs with the onset of hypoxia. Similar observations have been made on other lower vertebrates where heat flow rate was reported to decrease by 70–85%. This suggests that in anoxia tolerant vertebrates some physiological functions are maintained during anoxia while others are reduced or suspended [11]. Insects show dramatic reactions to anoxia and they are subject to a rapid breakdown of functions [13]. The heat flow rate is reduced to 5% or less of the normoxic value. The different reactions to anoxia in lower vertebrates and insects suggest that the energy state of the tissues is differently affected by anoxia. This has

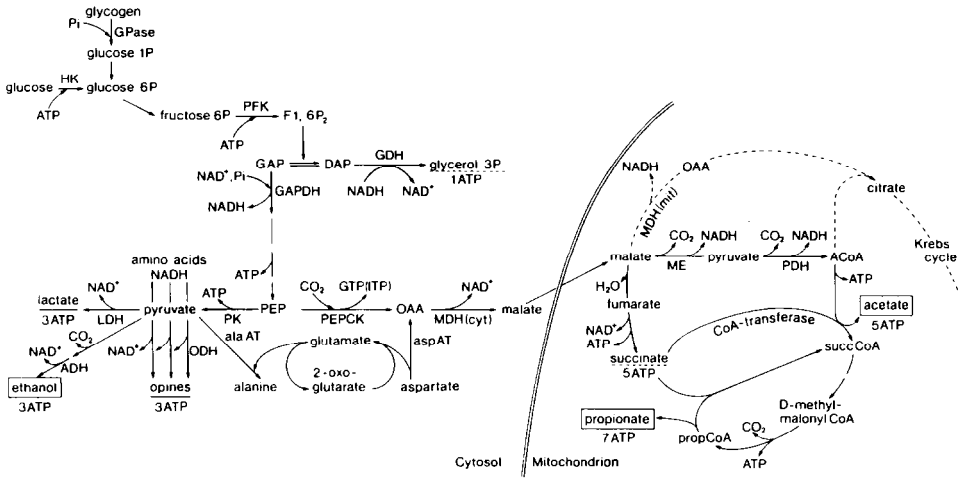


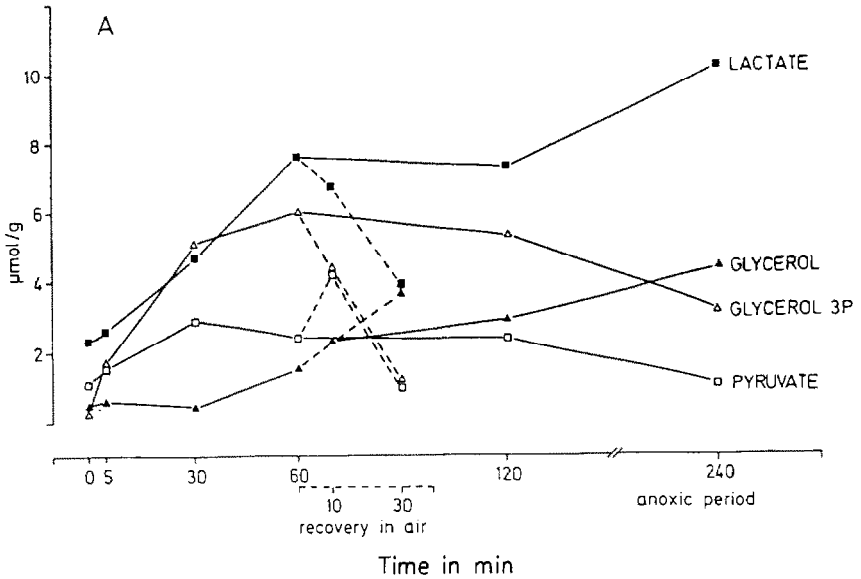
Fig. 4. A simplified general scheme of anaerobic energy metabolism as derived from work on various invertebrates and vertebrates. In vertebrates the anaerobic metabolism is restricted to carbohydrate as virtually the only substrate and lactate is the main end-product (classical anaerobic glycolysis). The same is true for adult insects, but insect tissues produce less lactate than vertebrate tissues, because they have a much lower activity of lactate dehydrogenase (LDH). Additional products such as glycerol 3-phosphate, glycerol and alanine are found in anaerobic insects (see Fig. 5). Many animals, especially invertebrates, that encounter hypoxia/anoxia in their natural habitats have evolved mechanisms to increase the yield of ATP and to form anaerobic end-products that can be excreted into the environment. In this scheme products of anaerobic metabolism are underlined (broken lines if they can be further metabolized anaerobically) and enclosed in boxes if they can be excreted. The yield of ATP is given with each product on the assumption that 1 glucosyl unit from glycogen is metabolized to yield only the respective end-product (from Wegener [1]).

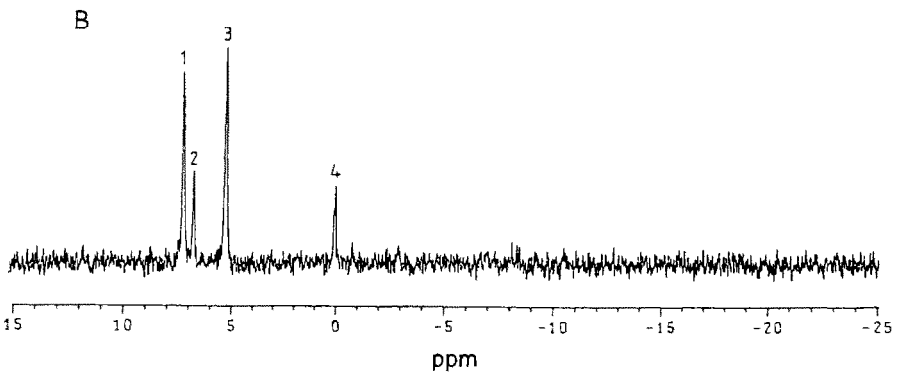
indeed been demonstrated. In the initial phase of anoxia, ATP contents and the cellular energy state in some organs of frog (such as skeletal muscle and heart [11]) are fully stabilized while ATP decreased with the length of the anoxic interval in other organs, most notably the brain [11].

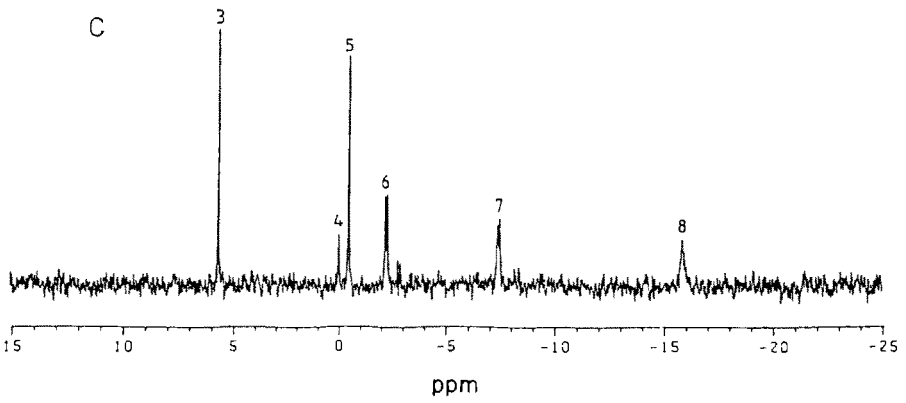
In anoxic insects the ATP contents and hence the cellular energy state is not stabilized in any organ. ATP appears to decline most rapidly in brain (by about 50% within 5 min of anoxia in locust brain and by 90% in the brain of the honey-bee [13, 22]). With longer anoxic intervals ATP will fall to levels that are almost undetectable.

Anoxic flight muscle loses ATP at a lower rate than anoxic brain, yet with prolonged anoxia (3–4 h) ATP will fall below 1% of the normoxic values in both *Locusta* and *Manduca* [23, 24]. This leads to the important conclusion that, at least in insects, the loss of almost all ATP does not necessarily cause cell death.

Much work has been devoted to the problem of which metabolic


A
LACTATE
GLYCEROL
GLYCEROL 3P
PYRUVATE
µmol/g
10
8
6
4
2
0 5
30
60
120
240
10
30
recovery in air
anoxic period
Time in min


B
1
2
3
4
15
10
5
0
-5
-10
-15
-20
-25
ppm


C
3
5
6
4
7
8
15
10
5
0
-5
-10
-15
-20
-25
ppm

pathways are operating during anaerobiosis in animal tissues. A variety of species of different systematic groups have been studied in this respect and a complex network of pathways has evolved from these studies (see Fig. 4). However, in vertebrates the only significant anaerobic pathway appears to be glycolysis with lactate as end-product. Lactate is rapidly accumulated in anaerobic vertebrate tissues, with the notable exception of some cyprinoid fish that are able to produce ethanol as glycolytic end-product which is excreted into the water [8, 25]. Insects have relatively low activities of lactate dehydrogenase, so that their capacity to reoxidize glycolytically produced NADH is small. Nevertheless, in adult insects anaerobic energy metabolism seems also to be restricted to glycolysis, although additional products may be formed such as alanine, glycerol 3-phosphate and glycerol (see Figs. 4 and 5). Overall, the production of anaerobic products, i.e. the metabolic activity during anoxia, is very modest in both *Locusta* and *Manduca* and this is in keeping with the markedly reduced anoxic heat flow rate.

The most striking difference between *Locusta* and *Manduca* was seen during recovery from anoxia, when *Locusta* produced heat at a rate markedly above the control rate for many hours whereas in *Manduca* the excess heat during postanoxic recovery was small and limited to less than 2 h.

The metabolic processes during anaerobiosis as reflected in changes in substrates, intermediates and products, are similar in *Locusta* and *Manduca.* Hence there is no indication as to anaerobic reactions in *Locusta* that could account for a large "oxygen debt". Our findings suggest that the integrity of cellular structures (for instance membranes) might be affected to different degrees in *Locusta* and *Manduca.* More research is required to identify the mechanisms that could account for the great differences in heat flow during postanoxic recovery in *Locusta* and *Manduca.*

Fig. 5. Products of anaerobic metabolism in insect organs (A, brain; B, C, flight muscle of the locust *Locusta migratoria*). A, Locust brain: anaerobic products accumulate to modest levels only, even if anoxia is prolonged. Glycerol 3-phosphate, lactate and glycerol are the main products. Metabolic recovery is rapid. B, ^{31}P NMR spectrum of a tissue extract from flight muscle of locusts that were subjected to 3 h of anoxia. Only three prominent peaks were observed and these were assigned to, peak 1, glycerol 3-phosphate, peak 2, AMP plus IMP, and peak 3, inorganic phosphate. Peak 4 is phosphocreatine which is not a compound occurring in insect muscle but was added to the extract as a chemical shift reference (ppm = 0.0). Note that no ATP and phosphoarginine, the phosphagen of insect tissues, are detectable in this spectrum. C, ^{31}P NMR spectrum of a flight muscle extract from locust that were subjected to 1 h of anoxia and were then allowed to recover for 8 h in air. This spectrum is not noticeably different from a normoxic control spectrum. Glycerol 3-phosphate, AMP and IMP are undetectable, while phosphoarginine (peak 5) and the three phosphorus atoms of ATP give rise to prominent peaks (peaks 6–8) (from Wegener [13]).

ACKNOWLEDGEMENT

Work from the authors' laboratory has been supported by grants from the Deutsche Forschungsgemeinschaft, D-53175 Bonn.

REFERENCES

1 G. Wegener, in H. Acker (Ed.), Oxygen Sensing in Tissues, Springer, Berlin, 1988, p. 13.
2 R. Rossen, H. Kabat and J.P. Anderson, Arch. Neurol. Psychiatry, 50 (1943) 510.
3 J. Ernsting, in J.A. Gillie (Ed.), A Testbook for Aviation Physiology, Pergamon, London, 1965, p. 270.
4 B.K. Siesjo, Brain Energy Metabolism, Wiley, New York, 1978.
5 G. Wegener, R. Michel and M. Thuy, Zool. Beitr., 30 (1986) 103.
6 J. Van Waversveld, A.D.F. Addink and G. van den Thillart, J. Exp. Biol., 142 (1989) 325.
7 J. Van Waversveld, A.D.F. Addink and G. Van den Thillart, J. Comp. Physiol. B, 159 (1989) 263.
8 G. van den Thillart and A. van Waarde, Mol. Physiol, 8 (1985) 393.
9 P.L. Lutz, M. Rosenthal and T.J. Sick, Mol. Physiol., 8 (1985) 411.
10 P.L. Lutz, Annu. Rev. Physiol., 54 (1992) 601.
11 G. Wegener and U. Krause, in P.W. Hochachka, P.L. Lutz, T. Sick, M. Rosenthal and G. van den Thillart (Eds.), Surviving Hypoxia: Mechanisms of Control and Adaptation, CRC, Boca Raton, 1993, Chap. 15.
12 G. Wegener, in A.P. Gupta (Ed.), Arthropod Brain: Its Evolution, Development, Structure and Functions, Wiley, New York, 1987, 369.
13 G. Wegener, in P.W. Hochachka, P.L. Lutz, T. Sick, M. Rosenthal and G. van den Thillart (Eds.), Surviving Hypoxia: Mechanisms of Control and Adaptation, CRC, Boca Raton, 1993, Chap. 28.
14 P.W. Hochachka, Mol. Physiol., 8 (1985) 331.
15 P.W. Hochacka and M. Guppy, Metabolic Arrest and the Control of Biological Time, Harvard University Press, Cambridge, MA, 1987.
16 C. Schulz, M. Thuy and G. Wegener, Thermochim. Acta, 187 (1991) 71.
17 H. Leivestad, Arbok Univ. Bergen, Mat. Naturvitensk. Ser., 5 (1960) 1.
18 D.C. Jackson, J. Appl. Physiol., 24 (1968) 503.
19 E. Gnaiger and I. Staudigl, Physiol. Zool., 60 (1987) 659.
20 I. Hardewig, A.D.F. Addink, M.K. Grieshaber, H.O. Pörtner and G. van den Thillart, J. Exp. Biol., 157 (1991) 143.
21 W. Engels, J. Insect Physiol., 14 (1968) 253.
22 S. Kieffer, Wirkung von Sauerstoffentzug (Anoxie) auf Verhalten und Stoffwechsel adulter Insekten. Eine vergleichende Untersuchung unter besonderer Berücksichtigung des Gehirnstoffwechsels, doctoral dissertation, Johannes Gutenberg University of Mainz, Federal Republic of Germany, 1988.
23 W. Weyel, T. Moratzky, A. Vierengel, H. Kolshorn and G. Wegener, Verh. Dtsch. Zool. Ges., 85 (1992) 176.
24 G. Burkhardt, Biochemische und biophysikalische Untersuchungen zur Regulation des Energiestoffwechsels in der Flugmuskulatur des Tabakschwärmers *Manduca sexta*, doctoral dissertation, Johannes Gutenberg University of Mainz, Germany, 1993.
25 E.A. Shoubridge and P.W. Hochachka, Science, 209 (1980) 308.