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IN VITRO METABOLIC DEPRESSION OF TISSUES FROM THE AESTIVATING FROG *NEOBATRACHUS PELOBATOIDES*

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Summary

The desert frog *Neobatrachus pelobatoides* reduced its resting metabolism *in vivo* by 60–70 % during 5–7 weeks of aestivation (summer dormancy). The rate of oxygen consumption ($\dot{V}_{O_2}$) of isolated and intact skeletal muscle, measured *in vitro*, was 70 % lower for aestivating frogs compared with non-aestivating frogs. The cause of the reduced $\dot{V}_{O_2}$ of aestivating frog muscle must lie in the tissue itself rather than being induced by external factors such as oxygen supply or blood-borne metabolites (because these were identical in the *in vitro* assay conditions), by any short-term effects produced by hormones (as these would have been washed out of the tissues during incubation) or by tissue dehydration (as the tissues from aestivating frogs had rehydrated to non-aestivating levels). The reduced *in vitro* muscle $\dot{V}_{O_2}$ accounted for 60–77 % of the frogs *in vivo* metabolic depression that accompanied aestivation. Other tissues of the aestivating frog, namely intestine, liver, skin and fat, did not have a reduced *in vitro* $\dot{V}_{O_2}$. We suggest that metabolic depression is initiated by reduced energy demand in cells and this consequently leads to reduced energy production.

Introduction

A great variety of animals, from insects to mammals, are capable of reducing their basal metabolism when faced with environmental stresses. This state of metabolic depression is usually characterised by a condition of dormancy, torpor or quiescence, where the basal metabolic rate is reduced by 60–90 %. This hypometabolic state extends survival time while the animal relies on stored fuel supplies for a period that may be several hours, overnight, a season, or several years.

The animal models that have been commonly used for the study of metabolic depression are those associated with cold (e.g. hibernating endotherms), anaerobiosis (e.g. intertidal molluscs, turtles and diving mammals) and dehydration (e.g. brine shrimp, aestivating snails, frogs and fish). Studies of these animals have

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provided insights into the common physiological problems of gas exchange and acid–base balance associated with some types of metabolic depression (Lahiri *et al.* 1970; Malan *et al.* 1981; Herbert and Jackson, 1985; Barnhart, 1989; Thomas *et al.* 1990). Biochemical studies have also revealed common themes. For example, adenylate levels are maintained in the tissues of the aestivating snail (Churchill and Storey, 1989) and in the brain of the anoxic turtle (Lutz *et al.* 1984; Chih *et al.* 1989) and therefore ATP depletion does not play a role in depressing metabolic rate. Similarly, ion gradients are maintained despite metabolic depression in the turtle brain (Sick *et al.* 1985; Chih *et al.* 1989) and the red cells of mammalian hibernators (Willis, 1987). Enzyme inhibition by phosphorylation or disaggregation accompanies metabolic depression in a wide range of animals, from molluscs to mammals (Storey, 1990; Brooks and Storey, 1990).

While the above studies have concentrated on the qualitative characteristics of metabolic depression and the biochemical correlates of depression, no studies have yet addressed the quantitative significance of the various tissues or metabolic processes in relation to the overall reduction in metabolic rate of the animal. For example, while enzyme phosphorylation has been shown to accompany metabolic depression, there are no data that enable us to gauge the possible contribution of the affected enzymes, pathways or tissues to the depression of metabolic rate. In addition, the extensive studies of the turtle brain have shown how this tissue is adapted to anoxia, but the brain accounts for less than 5 % of the oxygen uptake of the resting turtle (Mink *et al.* 1981) and so quantitatively can only play a minor role in overall metabolic depression. Kelly and Storey (1988) show that in the turtle subjected to anoxia the depression of glycolytic rate is greater in the liver than other tissues, but the liver is only 2–4 % of the total body mass and thus its contribution to overall metabolic depression is probably minimal.

Desert frogs are a useful model of metabolic depression. Many species survive extended dry periods by burrowing and aestivating; a number of species also form a cocoon of shed skin while in aestivation. A dry period can last from 6 months to several years and during this time the aestivating frog must survive on its own water and energy stores. In the laboratory these frogs are easily induced to aestivate by keeping them without water while protecting them from excessive dehydration. We have used the Australian desert frog *Neobatrachus pelobatoides* (Werner), a cocoon-forming and burrowing frog, to quantify tissue contributions to metabolic depression. The oxygen consumption of *N. pelobatoides* can be depressed by up to 85 % of the resting rate, but little is known about this metabolic depression at the molecular level apart from changes in enzyme associations in the brain during aestivation (Flanigan *et al.* 1990). In these frogs, metabolic depression is not associated with any obvious perturbation of internal homeostasis such as temperature change or anoxia. Oxygen is not likely to be limiting to the aestivating frog in its burrow (Seymour, 1973*b*). Cocoon development has the potential to induce hypercapnia and acidosis (Loveridge and Withers, 1981), but the onset of metabolic depression takes 2–4 weeks, during which time the cocoon is only beginning to form and is not yet a significant barrier to

water loss or gas exchange. Temperature, hypoxia, hypercapnia and tissue acidosis are therefore not likely to be signals for the onset of metabolic depression in *N. pelobatoides*. We have capitalised on this system and examined the tissues of the frog for metabolic depression *in vitro*, in the absence of such external signals as low temperature and low oxygen tension. An *in vitro* depressed metabolic system is useful *per se* as a model for the study of intrinsic metabolic depression, and also allows the quantification of tissue contributions to metabolic depression.

Materials and methods

Collection and maintenance of frogs

N. pelobatoides were collected in the southwest region of Western Australia, during July. Within a day of collection they were weighed with the bladder empty (=collection mass) and randomly allocated to a control (i.e. non-aestivating) or aestivating group. All frogs were kept separately in a plastic container, in a cupboard with an open container of water to maintain high local humidity, at a temperature of 15–20°C. Each control frog was kept with free distilled water and wet paper towelling, which was replaced daily. All experiments on the control frogs were completed within 3 weeks of capture, during which time they were not fed.

Aestivating frogs were maintained without free water. Initially moist paper towelling was placed in the containers. This was allowed to evaporate to dryness, so that the frogs slowly dehydrated and entered into aestivation. The containers had a lid with a small hole to reduce evaporative water loss from the frog but allow sufficient gas exchange to maintain atmospheric O_2 levels. The aestivating frogs were allowed to rehydrate and fill their bladders prior to aestivation. This was to offset the effects of dehydration at the start of aestivation, when the frog has not yet formed a cocoon to protect itself from evaporative water loss. They were left undisturbed until assayed, 5–7 weeks after the initiation of aestivation.

Frog metabolic rate

To measure oxygen consumption, the frogs were weighed (=final mass), then placed in a 500 ml air-tight chamber flushed with room air. They were left overnight for 10–17 h (the longer times for aestivating frogs), undisturbed in the dark. An air sample was withdrawn the next morning through an air-tight tap into a 50 ml syringe. The oxygen concentration of this sample (dry, CO_2-free) was measured by injection through a Servomex model 574 paramagnetic O_2 analyser. From this the rate of oxygen consumption (ml O_2 h^{-1}, STPD) was calculated. The collection masses of the frogs were used to calculate the mass-specific $\dot{V}_{O_2}$ (oxygen consumption (ml O_2 h^{-1} g^{-1}). This mass represents the mass of a hydrated frog with the bladder empty. Any subsequent mass loss due to starvation was considered to be negligible (see Results, *Frog masses*).

Tissue metabolic rates

Following measurement of the rate of oxygen consumption, the frogs were cranially pithed and the heart exposed for blood collection. The frogs were then spinally pithed and tissues were removed for *in vitro* $\dot{V}_{O_2}$ measurement. The tissues assayed were the small intestine, large intestine, liver, the pelvic patch ventral skin, abdominal fat body (when present) and the iliofibularis muscle. Tissues were placed in Ringer's solution immediately upon removal and kept at 25°C until assayed. The Ringer's solution contained 110 mmol l^{-1} NaCl, 3.4 mmol l^{-1} KCl, 2 mmol l^{-1} $CaCl_2$, 2.4 mmol l^{-1} $NaHCO_3$, 0.5 mmol l^{-1} $NaCH_3COOH$ and 5 mmol l^{-1} glucose. The Ringer's solution had a weak buffering capacity and was adjusted to an approximate pH of 7–7.4 at 25°C with HCl. We had previously determined that the metabolic rate of the tissues did not decline for up to 12 h when kept in Ringer's solution at 25°C, with the exception of the small intestine, which was therefore assayed first. Muscle was usually assayed last, 8–12 h after removal.

The tissue $\dot{V}_{O_2}$ was measured with a Strathkelvin Instruments oxygen meter (model 781), using an air-tight respiration cell (model RC 300) and a chart recorder. The tissues were placed in the cell with 1–2.5 ml of fresh Ringer's solution at 25°C. In order to use volumes larger than 1 ml in the chamber, the probe holder was used without the collar knob. It was necessary to choose an appropriately sized chamber to maintain a tight fit. The solution was mixed by a magnetic spinbar located under a plastic mesh, to prevent the stirrer disrupting the tissues.

The preparation of tissues for assay was as follows. The intestines were everted, and the small intestine cut into lengths of about 2 cm. The liver was cut into 250 μm slices using a McIlwain tissue chopper; a maximum of 10 slices was placed in 2 ml of Ringer's solution. Approximately 1 cm^2 of skin was cut and tied to a mesh so that it did not curl at the edges. The muscle was tied intact to a plastic frame at resting length. In order to tie the muscle without damaging or cutting the muscle fibres, the intact iliofibularis was excised by cutting through a piece of bone attached at the distal end and a small section of another muscle at the proximal end. These end attachments were used to tie the iliofibularis to the frame, and any excess end pieces were cut off before measurement. After assaying, the iliofibularis was removed and the $\dot{V}_{O_2}$ of the remaining pieces, still tied to the frame, was measured. The difference between these two measurements was taken to be the $\dot{V}_{O_2}$ of the iliofibularis muscle. The iliofibularis from each leg and at least two samples of skin and liver slices were assayed. After assay the tissues were weighed, freeze dried, and weighed again to determine dry mass and hence water content. The masses of any remaining liver, skin, fat body and intestines of the frog were also measured to determine the total mass of these tissues.

The oxygen consumption of the oxygen electrode in Ringer's solution was measured either prior to assay of a tissue or by removing the tissue from the system and continuing the assay. The blank was subtracted from tissue values. The magnitude of the blank reaction was significant for the small and less-active

tissues, in particular abdominal fat, being up to one-third of the tissue rate. Filtering the Ringer's solution through a 0.22 μm Millipore filter, cleaning the electrode and sterilising the plastic meshes and electrode chamber with 70% ethanol minimised the blank oxygen consumption, suggesting that part of it was due to bacterial contamination.

The proportion of metabolism that each tissue contributed to the total oxygen consumption was calculated according to the equation:

$$\% \text{ contribution} = 100 \times [\text{tissue } \dot{V}_{O_2} (\text{ml } O_2\, g^{-1} h^{-1}) \times \text{entire tissue mass (g)}] \div \text{total frog metabolic rate (ml } O_2\, h^{-1}).$$

The entire mass of the skeletal muscle was assumed to be 35% of each frog's collection mass (Putnam, 1979).

Results

Frog masses

There was no significant difference between the average collection masses of the control and aestivating frog groups (Table 1), so it is possible to compare the $\dot{V}_{O_2}$ of the two groups without adjusting for mass differences, and to compare the total oxygen consumption of frogs (ml $O_2\, h^{-1}$ whole frog^{-1}) between the two groups (Table 1). The control frogs did not lose mass when they were kept with access to free water. In contrast, the aestivating frogs, kept without water, did lose a significant amount of mass, most of which would be due to dehydration. We have calculated that any mass loss of the frogs due to starvation is negligible over a short period. A control frog would use 0.3 g of lipid in 3 weeks while an aestivating frog would use 0.3 g of lipid in the first 7 weeks of aestivation. We concluded, therefore, that any mass difference between capture and final masses was mainly due to water content; bladder content in the case of the control frogs and dehydration in the case of aestivating frogs. As a result, the capture masses of the frogs were used in all calculations that involved frog mass.

Table 1. *Mass (collection and final) and oxygen consumption rates of control and aestivating* Neobatrachus pelobatoides

	Mass (g)		Total $\dot{V}_{O_2}$ (ml $O_2\, h^{-1}$)	$\dot{V}_{O_2}$ (ml $O_2\, g^{-1} h^{-1}$)
	Collection	Final		
Control	8.16±0.30	8.32±0.34	1.05±0.18	0.125±0.018
Aestivating	8.70±0.90	6.46±0.48*	0.34±0.05**	0.043±0.009**

All values are mean±S.E. Control group $N=10$; aestivating group $N=6$.

* Final mass is significantly different from the collection mass in the aestivating group by paired Students t-test ($P \leq 0.05$).

** Aestivating oxygen consumption rate is significantly different from control value by Students t-test ($P \leq 0.01$).

Frog metabolic rate

Aestivating frogs were using 66 % less oxygen than the control frogs after 5–7 weeks of aestivation (Table 1). It can be difficult to measure the true resting metabolic rate of a frog, as any activity by the frog will significantly increase its oxygen consumption rate. A few of the control frogs had oxygen consumption rates that were much higher than the others, an indication that they were active during measurement. Previous measurements of control metabolic rate of *N. pelobatoides* reported a $\dot{V}_{O_2}$ of $0.113\,ml\,O_2\,g^{-1}\,h^{-1}$ with an average frog mass of 7.44 g (Flanigan *et al.* 1990). This corresponds to a $\dot{V}_{O_2}$ of $0.110\,ml\,O_2\,g^{-1}\,h^{-1}$ for a 8.16 g frog (i.e. mass adjusted to the average mass of the control group in this study), which is 12 % lower than the control $\dot{V}_{O_2}$ obtained in this study. This previous value can be considered to be a more accurate measurement of the resting oxygen consumption of *N. pelobatoides* as it involved repeated measurements of each frog, with the lowest of these taken as the true resting metabolic rate. If this lower control $\dot{V}_{O_2}$ is used, then the aestivating frogs depressed standard metabolism by 60 %. Time considerations made repeated measurement impracticable for this study. Since aestivating frogs are reluctant to move, the problem in $\dot{V}_{O_2}$ measurement does not occur in this group.

Tissue in vitro *metabolic rate*

The *in vitro* $\dot{V}_{O_2}$ of the tissues from control and aestivating frogs are shown in Fig. 1A (small and large intestine) and Fig. 1B (muscle, liver, skin and fat) and compared with the *in vivo* metabolic rate. Muscle is the only tissue that had a significantly reduced *in vitro* $\dot{V}_{O_2}$ in the aestivating frog, being 70 % lower than the control level.

The tissues of aestivating frogs are normally dehydrated compared to control (hydrated) levels (Flanigan *et al.* 1990). In this study, however, it was found that the water content of the incubated tissues of aestivating frogs where the same as those of the control tissues (data not shown), indicating that they had rehydrated during incubation in the Ringer's medium. As a result, the $\dot{V}_{O_2}$ of control and aestivating tissues are compared on a wet mass basis. Comparison of tissue $\dot{V}_{O_2}$ on a dry mass basis shows the same change in the muscle and no change in the other tissues. The water contents of the control frog tissues after incubation in the Ringer's solution are the same as those measured in a previous study where the water content was measured in the tissues directly upon removal (Flanigan *et al.* 1990), indicating that the tissues from hydrated frogs had not absorbed any additional water during incubation.

The contribution of each tissue to total body mass and of the *in vitro* tissue $\dot{V}_{O_2}$ to the total *in vivo* oxygen consumption of a frog (Table 2) indicates that we have accounted for 50 % of the mass and 80 % of the metabolic rate of the control frog. Muscle is the highest contributor, accounting for 65 % of metabolic rate of the control frogs and 50 % of the metabolic rate of aestivating frogs. It can be seen in Fig. 1B that the metabolic rate of muscle *in vitro* is similar to that of the whole animal for both control and aestivating frogs.

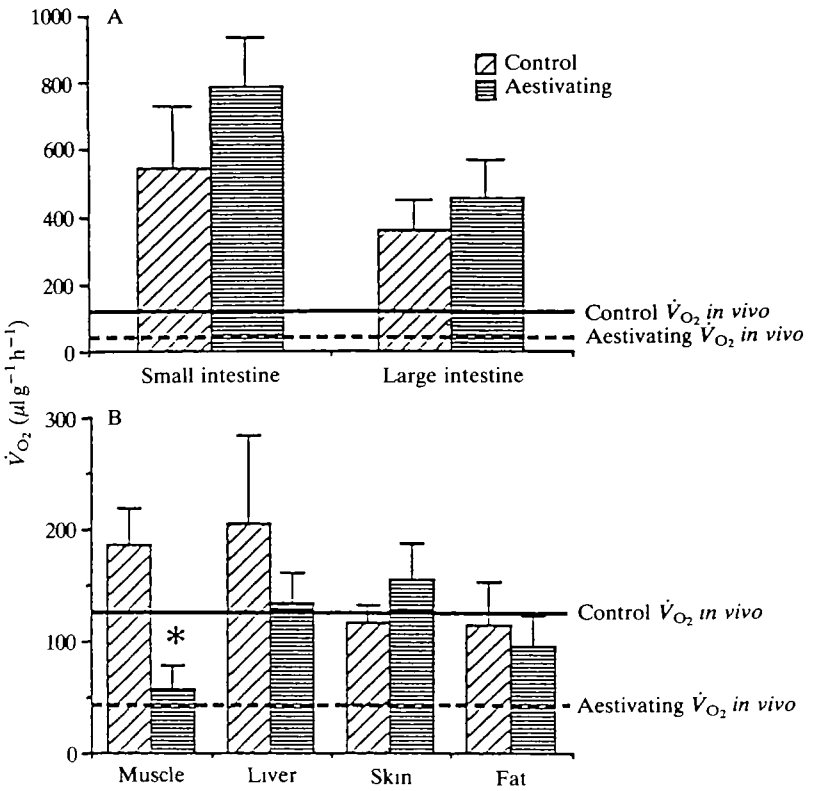

Fig. 1. The *in vitro* $\dot{V}_{O_2}$ (μl O_2 g^{-1} h^{-1}) of (A) small and large intestine, and (B) muscle, liver, skin and fat from control and aestivating *Neobatrachus pelobatoides*. Values are means±S.E. Horizontal lines are the oxygen consumption of the intact animals (μl O_2 g^{-1} h^{-1}). For control tissues N=11, except for muscle and fat where N=7. For aestivating tissues N=6, except for fat where N=4. * Significantly different from the control value by Students t-test (P=0.01).

Discussion

Aestivating amphibians show a pronounced metabolic depression. The resting $\dot{V}_{O_2}$ is reduced during aestivation to 21 % of the resting value in *Lepidobatrachus llanensis* (McClanahan *et al.* 1983), 22 % in *Scaphiopus conchii* (Seymour, 1973*a*), 27 % in *Pyxicephalus adspersus* (Loveridge and Withers, 1981) and 30 % in *Cyclorana platycephalus* (van Beurden, 1980). The reduction in $\dot{V}_{O_2}$ reported in this study for *N. pelobatoides*, to 34 % of resting, is similar to that reported previously (Flanigan *et al.* 1990). The marked metabolic depression of these aestivating frogs dramatically extends their maximum possible duration for aestivation. For example, it has been calculated that aestivating *C. platycephalus* would be able to survive for up to 7 years on their fat pad energy stores (van Beurden, 1980), but this would be reduced to about 2 years if there were no metabolic depression.

Table 2. *Tissue contribution to frog mass and metabolic rate for control and aestivating* Neobatrachus pelobatoides

Tissue	Percentage of total hydrated mass	Contribution to metabolic rate (%)	
		Control	Aestivating
Muscle	35*	65±18 (6)	51±21.5 (6)
Skin	8.3±0.38 (17)	9.9±1.7 (9)	28±5.5 (6)
Liver	1.8±0.12 (17)	3.4±0.99 (8)	6.5±1.8 (6)
Small intestine	0.53±0.08 (17)	2.4±0.60 (9)	5.4±1.2 (6)
Large intestine	0.28±0.04 (17)	0.90±0.19 (9)	1.5±0.34 (6)
Fat	0.34±0.11 (12)	0.25±0.08 (7)	0.48±0.16 (4)
Unmeasured tissues	53.7	18.2	7.1
Total	100	100	100

*From Putnam (1979). Other values are mean±S.E. (*N*).

No previous studies have quantitatively addressed the contributions of different tissues to the overall metabolic depression. We have found that the *in vitro* $\dot{V}_{O_2}$ of muscle was 70% lower in aestivating frogs whose metabolic rate was 60–70% lower than control frogs. Because muscle was usually the last tissue to be measured, the mechanism of metabolic depression must have been stable for up to 12 h after removal of the tissues. As this reduction was manifested in an *in vitro* system, the mechanism of this reduction presumably must lie in the cells themselves, and not in external factors such as oxygen supply or blood-borne metabolites, since these factors are constant in the *in vitro* system. The mechanism could be hormonal, but the process would have to be stable enough to last for 8 h of *in vitro* incubation in the Ringer's solution, during which time any free hormone would be washed out and diluted, or broken down. The muscle and the other tissues had rehydrated before measurement of $\dot{V}_{O_2}$, so short-term effects of dehydration in the tissue itself can be excluded as a cause of the reduced oxygen consumption.

The reduction in the oxygen consumption of muscle can account for a major proportion of the metabolic depression in the aestivating frogs. After 5 weeks of aestivation, the frogs were consuming on average $0.71\,ml\,O_2\,h^{-1}$ less than the control frogs (Table 1). At the same time, the oxygen consumed by the entire muscle mass declined from an average of 0.58 to $0.15\,ml\,O_2\,h^{-1}$, i.e. a decline of $0.43\,ml\,O_2\,h^{-1}$ (based on the average of each frog's total muscle oxygen consumption). Hence the reduced muscle $\dot{V}_{O_2}$ would account for 60% of the metabolic depression that occurs in the aestivating group. If the more accurate resting metabolic rate of *N. pelobatoides* obtained in a previous study is used (see Results, *Frog metabolic rate*), then the total oxygen consumption of the control group from the present study is $0.90\,ml\,O_2\,h^{-1}$. In this case, the oxygen consumption is reduced by $0.56\,ml\,h^{-1}$ from the control level, and the reduced muscle $\dot{V}_{O_2}$ can account for 77% of metabolic depression. The accuracy of this estimate

depends on two major assumptions. One is that muscle mass is taken as being 35 % of body mass. In Putnam's (1979) study the muscle content of three species of anurans ranged from 25 to 39 %. The other assumption is that the iliofibularis muscle is representative of the resting $\dot{V}_{O_2}$ of all the frog muscle. In isolated and intact muscle from the hamster the $\dot{V}_{O_2}$ varied by up to 25 %, according to the oxidative capacity of the muscle fibres (Sullivan and Pittman, 1984). If we were to assume that *N. pelobatoides* is only 25 % muscle and that the average muscle $\dot{V}_{O_2}$ is 25 % lower than the iliofibularis rate, then reduced muscle $\dot{V}_{O_2}$ would account for only 40 % of metabolic depression. This assumption also means that muscle accounts for only 35 % of the total metabolic rate in the control frogs. In contrast, if the average muscle $\dot{V}_{O_2}$ was 25 % higher than that of the iliofibularis then the reduced muscle $\dot{V}_{O_2}$ would account for nearly 100 % of the metabolic depression. In the same circumstances, muscle would account for 80 % of the control metabolic rate. The true contribution lies somewhere in between but it is apparent that the reduced $\dot{V}_{O_2}$ of muscle plays a major part in overall metabolic depression.

The assumption that the $\dot{V}_{O_2}$ of a muscle measured *in vitro* is a true measure of the resting *in vivo* $\dot{V}_{O_2}$ is essential to the above interpretations, so some justification of this assumption is required. The *in vivo* $\dot{V}_{O_2}$ can be determined by measuring blood flow rate and O_2 content of arterial and venous blood. In the rat hindlimb, which is mostly muscle, the $\dot{V}_{O_2}$ determined by this method is 0.53 ml $O_2\,g^{-1}\,h^{-1}$ at 37 °C (Ruderman *et al.* 1971). Similar values, i.e. within 30 %, have been obtained in perfused rat hindlimb (Honig *et al.* 1971; Ruderman *et al.* 1971; Richter *et al.* 1984) and in small isolated intact muscles of the rat and hamster (Wardlaw, 1986; Sullivan and Pittman, 1984). The similarity of muscle metabolic rate measured *in vivo* and *in vitro*, and the fact that Table 2 seems to be a realistic measure of tissue contribution to metabolic rate, leads us to conclude that the *in vitro* $\dot{V}_{O_2}$ of muscle and other tissues is a reliable measure of *in vivo* $\dot{V}_{O_2}$.

Muscle is the main contributor to the total resting metabolic rate (Table 2). Consequently, for any mechanism to reduce metabolic rate and have a quantitative significance it must occur in muscle. In contrast, if muscle were to account for only 65 % or less of the total resting metabolic rate, then significant metabolic depression of the other tissues would be required for deep aestivation where the total $\dot{V}_{O_2}$ declines to less than 35 % of the resting rate. Although we have determined that muscle is the major site of metabolic depression in the aestivating frog, we cannot assume that it is the only site of metabolic depression. Since only 77 % of total metabolic depression is accounted for, there is the possibility that other tissues may have been depressed *in vivo* but in a manner that did not manifest itself in the *in vitro* assay system or were depressed by a less stable mechanism and hence were not depressed when assayed. After muscle, skin makes the next most significant contribution to metabolic rate (other tissues having only a minor role) and so skin may have to be depressed in deep aestivation.

In Table 2 it appears that the 'unmeasured tissues' are depressed *in vivo*. It is

more likely, however, that the contribution of these tissues to the control metabolic rate was underestimated to some degree because of overestimation of the resting metabolic rate of a few control frogs, presumably due to activity. This means that the 'unmeasured tissues', which account for 50 % of the mass of the frog, would actually contribute less than the 18.2 % of control metabolic rate that is indicated in Table 2. The $\dot{V}_{O_2}$ of liver may, additionally, be underestimated owing to damage from slicing. Furthermore, the large variation that occurs in the estimates of the tissue contributions (especially of muscle) to metabolic rate markedly affects the accuracy of the value for total unaccounted $\dot{V}_{O_2}$. Therefore, while the data make it appear that the 'unmeasured tissues' of the frog have a depressed metabolism, this may not actually be the case. Aestivating frogs may show a more accurate value for the 'unmeasured tissues' because the measurement of frog metabolic rate in this group is less likely to be affected by frog activity.

It is probable that the unmeasured tissues (heart, bone, blood, brain, connective tissue, etc.) may only account for a small proportion of the frog's oxygen consumption. The central nervous system of an amphibian contributes approximately 5 % of resting metabolism (Mink *et al.* 1981). Organs like the heart and kidney make up a small proportion of the body mass but may make a noticeable contribution to metabolic rate; in man, the kidney contributes 7 % and the heart 11 % to the total oxygen consumption. Blood, bone and connective tissue, which make up a substantial proportion of the 'unmeasured' body mass contribute in total less than 5 % of the metabolic rate in man (McGilvery and Goldstein, 1983).

The mechanism for metabolic depression is not clear from this study, although it is possible to exclude some short-term factors. The reduced metabolic state must be accompanied by a reduction in ATP turnover, but the energy-supplying processes of a cell cannot be reduced in the long term without a commensurate reduction in energy usage. If the organism is to avoid the consequences of severe energy depletion (which occurs in anoxia-sensitive tissues when subject to anoxia), i.e. reduction in energy charge, dissipation of ion gradients and finally cell death (Hochachka, 1986), then the rates of energy-using processes in the cell must first be reduced, and metabolic pathways will undergo normal (adenylate and metabolite based) regulation to reduce energy production. This would produce regulatory effects such as enzyme phosphorylation (Storey, 1990), but these effects would be a consequence, not a cause, of metabolic depression.

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