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Metabolism during asphyxia: a revision

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1. Introduction: Diving metabolism

In diving birds and mammals the "on-board" oxygen stores at the start of a forced, breath-hold dive will be insufficient to provide for more than a small proportion of the maximum underwater endurance if aerobic metabolism continues at the same rate as before submergence. However, a suite of adjustments consisting of apnoea, bradycardia and redistribution of blood flow ensures survival by restricting the use of the circulatory and respiratory oxygen store solely to oxygen sensitive tissues such as the heart and brain (Scholander, 1940). Hence, as Andersen (1959) showed by measuring pulmonary oxygen levels during submergence, diving aerobic metabolic rate falls to less than 5% of the pre-dive rate.

Blood flow redistribution means that large areas of the body are hypoperfused and, when tissue oxygen stores are exhausted, must metabolise anaerobically. The question as to whether the shortfall in aerobic metabolism is fully compensated by increased anaerobic metabolism has yet to be answered. Nevertheless, it has certainly been addressed, and current views would suggest that whole body metabolic rate declines during submergence due to a partial or absolute depression of ATP (adenosine-5′-triphosphate) turnover in hypoperfused tissues (Hochachka and Guppy, 1987).

There is both indirect and direct evidence to support this view. The most persuasive indirect evidence comes from measurement of the post-dive oxygen debt (Scholander, 1940). On many occasions the excess of post-dive oxygen consumption over the pre-dive rate is insufficient to provide for maintenance of a pre-dive metabolic rate if the oxygen debt is averaged over the dive, indicating a reduction in metabolism. However, in animals known to struggle violently during forced submergence the excess post-dive oxygen uptake is sufficient to provide for an elevated level of in-dive metabolism (Scholander, 1940; Fairbanks and Kilgore, 1978). This, combined with the fact that it is not known what proportions of the post-dive lactate appearing

in blood are removed by oxidation and by gluconeogenesis, means that the size of the oxygen debt is not a solid indication of events during diving.

Direct calorimetry, on the other hand, should provide a clear indication of metabolic events in a dive. However, even this method is not without its problems because the animal must be in a "steady-state" with heat loss equalling heat gain. Further, there are complications caused by the fact that heat produced by metabolism over short diving time periods (5–6 min) is only a small proportion of the total heat content of the animal. Consequently, Pickwell (1968) using a simple calorimeter to measure heat loss from a diving duck, compensated by subtracting immediate post-death levels of heat loss from those obtained in a dive. Using this manipulation, Pickwell (1968) claimed that heat production in a dive might be as low as 5 % of the pre-dive metabolic rate, a level of metabolism that could be supported aerobically! Hence, profound metabolic depression or even metabolic arrest was indicated in the hypoperfused tissues.

2. *Metabolism in hypoperfused muscles*

Muscle, which makes up about one-third of the total body mass, is the major hypoperfused tissue in forced dived homeotherms. We looked at duck pectoral muscle metabolism during diving using ^{31}P nuclear magnetic resonance spectroscopy (NMRS) (Stephenson and Jones, 1992). Spectra (Fig. 1) were used to calculate ATP synthesis from hydrolysis of phosphocreatine (PCr) during submergence. Also, the change in intracellular pH allied to measures of intracellular buffering capacity of muscle, were used to calculate lactate production and therefore ATP synthesis from anaerobic metabolism (Fig. 2). These estimates of diving ATP synthesis must equal ATP utilisation, since ATP levels were unchanged.

ATP synthesis from stored oxygen, combined with myoglobin, is minor. Furthermore, it would be expected that intracellular oxygen would be utilised early in the dive. Dive times averaged 6.5 min and in that period, PCr declined by 22 % from pre-dive values. ATP synthesis from hydrolysis of PCr averaged 1.15 $\mu mol \cdot min^{-1} \cdot g^{-1}$ which is somewhat above the resting rate of ATP utilisation in duck muscle. Since 78 % of PCr remained after 6.5 min submergence then it is possible for PCr to power resting metabolism for nearly 30 min of submergence. The maximum dive time ever recorded for an adult duck is 25 min (Richet, 1899).

ATP production from lactate, using Pörtner's (1990) analysis of intracellular buffering capacity, was estimated at 2.6 $\mu mol \cdot min^{-1} \cdot g^{-1}$. This stimulation of glycolysis ("Pasteur effect") during anoxia represents an enormous in-

crease in flux rate through the Embden-Meyerhof-Parnas (EMP) pathway. At rest there is an ATP yield of 0.9 $\mu mol \cdot min^{-1} \cdot g^{-1}$ which means that only 0.025 $\mu mol \cdot min^{-1} \cdot g^{-1}$ glycosyl units (i.e. glucose-1-phosphate from glycogen) are channelled through the EMP pathway since 36 µmol TP are yielded from each µmol glycosyl unit. However, during anaerobiosis only 3 µmol ATP $min^{-1} \cdot g^{-1}$ are obtained from each µmol glycosyl unit. Therefore at end-dive 0.87 $\mu mol \cdot min^{-1} \cdot g^{-1}$ glycosyl units must be channelled through the EMP pathway which corresponds to a 35 times increase in flux rate. Hence a "negative Pasteur effect", which is assumed to spare energy stores (Hochachka 1985, 1988; Storey 1985), is not part of the metabolic arsenal of anoxic duck muscle.

Therefore, in hypoperfused muscle there is no reduction in metabolism from the resting level. In fact, ATP synthesis and utilisation increases by 3—4 times the resting rate and flux rates increase enormously. This is not unexpected since many ducks struggle during forced diving, and Scholander (1940) showed that higher post-dive oxygen debts are incurred by animals that are active during submergence. Hence, as muscle is one-third of body mass and muscle metabolism increases 3—4 times then, on a whole body

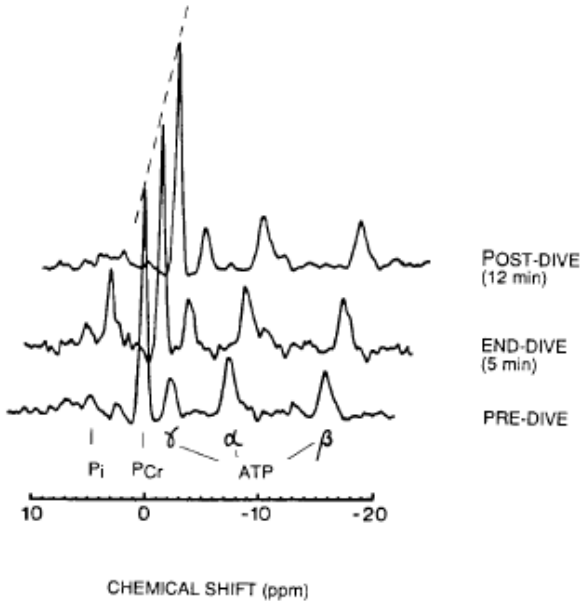

Fig. 1: ^{31}P spectra obtained from the pectoral muscle of a single Pekin duck before, during and after a forced dive. Each spectrum is the sum of 64 scans acquired in two minutes which bracket the time shown. Spectra are stacked in chronological order from bottom left to top right and each spectrum is displaced to the right by 1.5 ppm. The chemical shift of phosphocreatine was arbitrarily set at zero.

basis and assuming no metabolism at all in other hypoperfused tissues, forced dive heat production should be in excess of the resting rate. This conclusion is in sharp contrast to the almost total reduction in heat output from metabolism estimated by Pickwell (1968) using direct calorimetry.

3. *Conflict between ^{31}P NMRS and direct calorimetry*

Some of the problems of performing direct calorimetry on animals that are not in a steady state, as occurs during forced diving, have been alluded to above. Nevertheless, the difference between ^{31}P NMRS and direct calorimetry for estimating diving metabolism is spectacular.

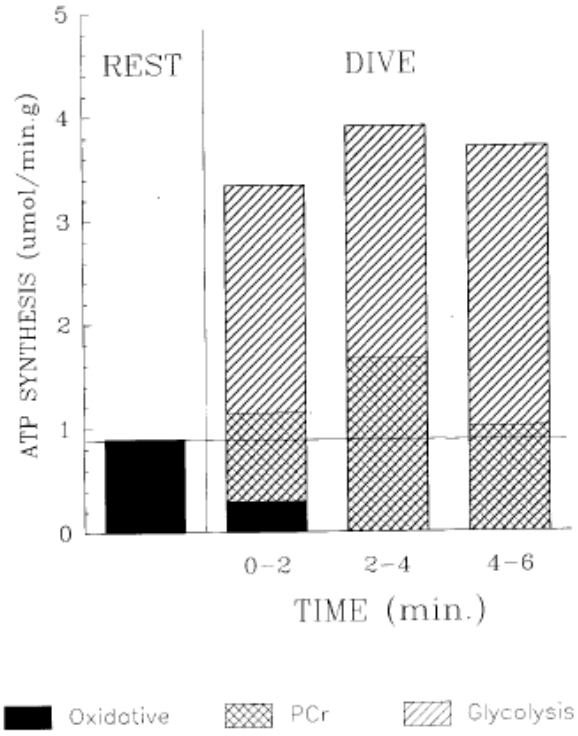

Fig. 2: Total ATP synthesis (and mode of production) in Pekin duck pectoral muscle at rest (all oxidative, filled) and at 3 time periods during forced dives. Oxidative metabolism (filled) was estimated from the myoglobin-bound oxygen store, assuming complete ischemia, and that the entire oxygen store of the tissue was used within the first two minutes of the dive. Glycolytic ATP synthesis (hatched) was estimated from the calculated net lactate (La^-) accumulation, assuming $ATP/La^- = 1.5$ in ischemic muscle. Calculations of glycolytic ATP synthesis were made assuming that the non-bicarbonate buffering capacity of the muscle was 28.8 $\mu Eq \cdot pH^{-1} \cdot g^{-1}$. Net PCr hydrolysis (cross-hatched) was derived from ^{31}P NMR spectra (from Stephenson and Jones, 1992).

Total heat loss of a duck, during a dive of 5–6 min duration, is only a small proportion of the total carcass heat content. Hence, to accentuate the change in metabolism during submergence Pickwell (1968), subtracted carcass heat loss rates from those measured during submergence. This certainly emphasised the reduction in metabolism during submergence but surely this manipulation begs the question. Since deep body temperatures are similar before and during diving then total carcass heat content is relatively unchanged by submergence. However, convective heat transfer to the body surface is reduced by cutaneous vasoconstriction so surface temperatures fall during submergence and heat loss will be slowed. This contributes to the lack of a steady state during forced dives with respect to heat loss which cannot be corrected by subtracting carcass heat loss from the dive heat loss.

Furthermore, the actual value taken for carcass heat loss will have a significant effect on estimates of diving heat production. Pickwell (1968) used

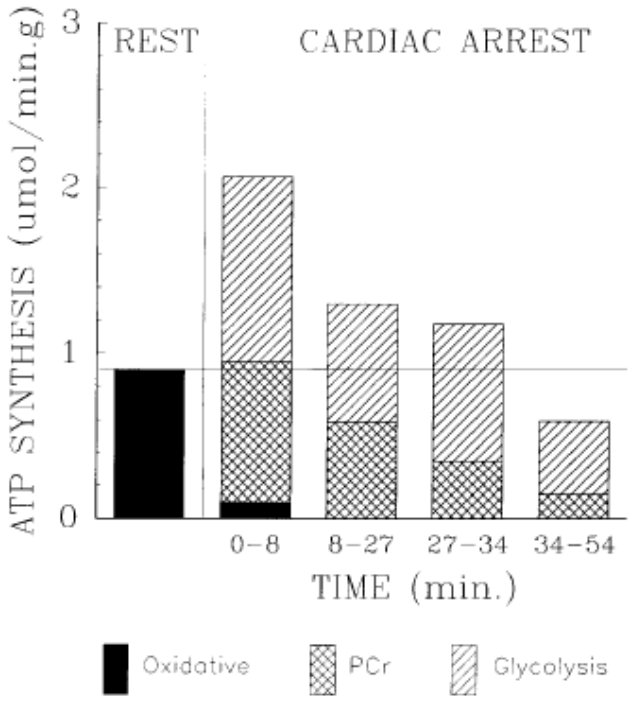

Fig. 3. Calculated rate of ATP synthesis in Pekin duck pectoral muscle, averaged over specified time intervals before and during ischemia induced by cardiac arrest. ATP generated by oxidative phosphorylation is indicated by the filled section of the bars. The estimates of resting ATP synthesis (horizontal dashed line) and ATP derived from the myoglobin-bound oxygen store were taken from Stephenson and Jones (1992). Glycolytic ATP synthesis (hatched) and ATP synthesis from PCr (cross-hatched) were obtained as described in Fig. 2 (from Stephenson, 1993).

onset of cardiac fibrillation as time of death, assuming that metabolism would cease within a couple of minutes. However, ^{31}P NMRS shows that metabolism, in terms of ATP synthesis and utilisation takes nearly 50 min to fall below the resting value (Fig. 3). Consequently, values for carcass heat loss obtained in the second hour after death, not the second minute, would give a more realistic estimate of heat loss in the absence of metabolism. Pickwell (1968) obtained values which were between 35 and 65 % of pre-dive heat production in the second hour after death. If carcass heat loss is assumed to be about 50 % of pre-dive heat loss (average of Pickwell's values) and is subtracted only from the diving value (after Pickwell, 1968) then diving metabolism appears to fall to one-third of pre-dive. On the other hand, if carcass heat loss is subtracted from both pre-dive and dive heat loss values then diving heat production is 70 % of pre-dive.

However Pickwell's (1968) data are manipulated, there is undoubtedly a substantial reduction in heat production during diving even in dives of 5—6 min duration, which is close to dive times used in NMRS studies. In contrast, the NMRS studies suggest that whole body metabolism should go up during diving, even if there is complete metabolic arrest in all tissues except the brain, heart and muscle.

4. Conflict between ^{31}P NMRS and direct calorimetry: a resolution

The majority of heat generated by the body is in production rather than utilisation of ATP. Obviously, ATP production is aerobic in the pre-dive period and anaerobic during submergence. If ATP can be produced at less enthalpy during diving than aerobically at rest then this could resolve the discrepancy between direct calorimetry and NMR. Woledge (1989) has shown ATP production from PCr releases only about 40 % of the heat that aerobic ATP production releases. Hence, in a quiet dive heat output would fall even through ATP turnover in muscle continued at resting levels from hydrolysis of PCr. In a similar vein, Gnaiger (1983) has shown that ATP produced anaerobically from breakdown of glycogen to lactate only releases about 60 % of the heat of aerobic ATP production from glucose. Hence, it is possible for ATP turnover to be considerably increased above resting levels without much change in heat production, if ATP is produced glycolytically.

Consequently, ^{31}P NMRS and direct calorimetry data taken together suggest that calorimetry values be taken at face value and not manipulated to account for carcass heat loss. Therefore, for forced dives by restrained animals it is probable that whole body heat loss is little changed from rest due to considerable increases in both flux rates and ATP synthesis in tissues such as

muscle. Hence, a revision of our views with respect to forced dive metabolism at both the whole body and individual tissue is indicated.

5. *Summary*

Aerobic metabolism is reduced by the exclusion of large areas of the body from access to the lung and blood oxygen store during asphyxiation of accomplished avian or mammalian divers. However, whether the fall in aerobic metabolism is compensated by a like increase in anaerobic metabolism is controversial.

A re-examination of published data of metabolic measures such as indirect and direct calorimetry allied with ^{31}P magnetic resonance spectroscopy of muscle metabolites indicates that there is not a major reduction of whole body metabolism during asphyxia.

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