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The Lactacid Oxygen Debt in Frogs after One Hour's Apnoea in Air

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Summary. 1. During one hour's apnoea in air curarised *Rana pipiens* showed a reduction in oxygen metabolism of some 21 %. When artificial ventilation was restarted the frogs consumed 178 μl more oxygen in the first 10 min than in comparable periods before the apnoea. Over the recovery period of one hour the frogs consumed 350 μl more oxygen than in the hour before the apnoea.

2. Blood lactic acid concentrations increased during apnoea and fell during the recovery period, the highest lactate levels being attained at the end of the apnoea and not during the initial part of the recovery period. The lactate: pyruvate ratio rose during apnoea. Blood glucose concentrations were above pre-apnoeic values in the early part of the recovery period.

3. After one hour's apnoea in oxygen, during which there was no significant reduction in oxygen metabolism, curarised frogs displayed no oxygen debt when artificial ventilation was restored.

4. The results of the present experiments are discussed in the light of previous data and it is concluded that, in normal frogs, the increase in oxygen uptake following submergence has three elements, the major one being the oxygen cost of increased activity in the form of hyperventilation.

Introduction

Curarisation of frogs has proved useful for investigation of both metabolic and circulatory effects of prolonged apnoea in air, as well as allowing assessment of the oxygen debt in the absence of activity (Jones, 1972b). When artificial ventilation is restarted after one hour's apnoea in air, frogs (*Rana esculenta*, average wt = 37.8 g) consume 110–130 μl more oxygen in the first 10 min than in comparable periods before the apnoea. This excess uptake is about equal to the amount of oxygen calculated to have been removed from the oxygen store of the blood during apnoea (Jones, 1972b). However, during a recovery period of one hour more oxygen is consumed compared with the pre-apnoeic period than can be accounted for by replenishment of oxygen stores. Analysis of acid-base balance of apnoeic frogs suggested that metabolic acids were being added to the blood during apnoea (Jones, 1972b), and it is possible that the more prolonged increase in oxygen

uptake throughout the recovery period is used to repay a lactacid oxygen debt.

Exposure of *R. pipiens* to 100 % N_2 results in depletion of ventricular and gastronemicus muscle and of hepatic glycogen concentrations with time, and iodoacetate poisoned frogs succumb in total anoxia much sooner than normal animals (Rose and Drotman, 1967). This work, with that of Armentrout and Rose (1971) in which blood sugar and lactic acid levels were measured in *Bufo cognatus*, gives a clear indication that anaerobiosis provides energy for maintenance of physiological integrity in anurans during total anoxia, but does not give any idea of the degree of anaerobiosis which might be expected to occur under conditions such as diving, when oxygen metabolism is maintained between 50–67 % of the pre-diving level (Jones, 1967).

Consequently in the present experiments an attempt has been made to measure the lactate and pyruvate changes undergone by curarised frogs (*R. pipiens*) during and after one hour's apnoea in air and to relate this to the extra oxygen consumed during recovery from apnoea.

Methods

The experiments were performed on 40 *R. pipiens* at 24° C. The frogs were obtained from commercial suppliers and acclimated to a temperature of $24 \pm 2°$ C for a period of not less than 30 days. During the period of acclimation the frogs were fed mealworms and the individual members of the population suffered no gross weight changes.

Twenty-three frogs were used to determine oxygen consumption in a respirometer at $24 \pm 0.05°$ C. Heart rate was also determined in twelve of these animals by means of copper wires sewn under the skin (Jones, 1970). A cannula (2 cm long and 2—3 mm diameter) was inserted into the tip of one lung under MS 222 Sandoz anaesthesia (300 mg/L), achieved by immersing the animal in the solution. After recovery from the anaesthesia the frogs were paralysed by injection of a solution of curare into the dorsal lymph sac (0.02 mg/g wet weight). A complete description of the experimental procedures and precautions taken to ensure accuracy when using the respirometer has already been given (Jones, 1970, 1972b). When the frogs were in the respirometer the lungs were ventilated using a variable displacement pump (Jones, 1970; rate 6–7/min, volume 1 ml/10 g wt) and apnoea was achieved by switching off the pump with the lungs collapsed. Oxygen consumption and heart rate were measured in 12 frogs before, during, and after one hour's apnoea in air and in 11 frogs these procedures were repeated in an atmosphere of pure oxygen.

Fifteen frogs were used to measure blood lactic and pyruvic acids. In these experiments the body temperature was maintained at $24 \pm 1°$C by placing the curarised animals, attached to a cork board, on a warming tray. Water at 24° C was added frequently to keep the skin moist. Before curarization a cannula was inserted into the lungs and a catheter (P. E. 50) into the sciatic artery under MS 222 Sandoz anaesthesia. Blood samples were withdrawn from the sciatic artery before, during, and after a period of one hour's apnoea in air. The sample size was 50 or 100 µl depending on the size of the frog. After collection the sample was fixed in

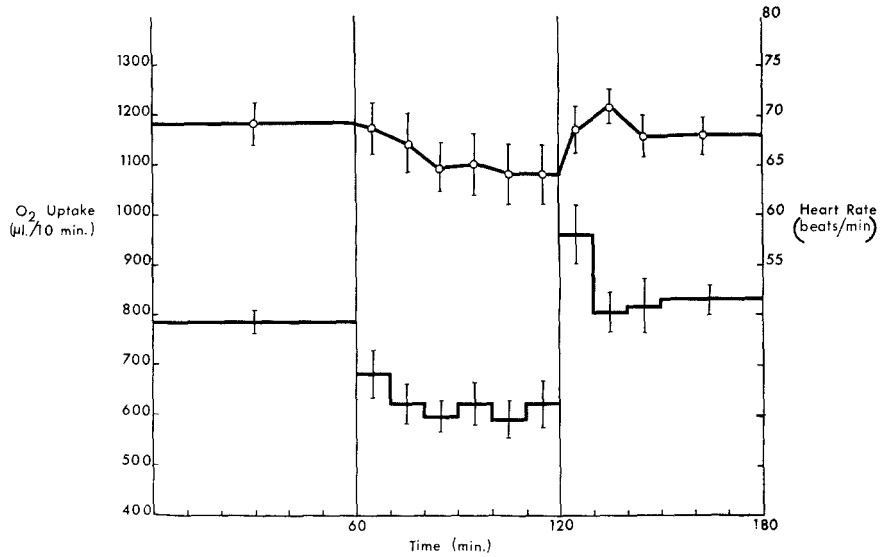

Fig. 1. Oxygen consumption and heart rate of curarised frogs before, during and after one hour's apnoea in air. The artificial ventilation was stopped at 60 min and restarted at 120 min. Average values of 12 experiments, average weight = 37.1 ± 1.8 g ($n = 12$). ○ heart rate

perchloric acid (8 %), shaken vigorously, and stored at —20° C for later analysis. The blood samples were analysed for pyruvic and lactic acid concentrations using methods described in Sigma Technical Bulletins No. 726-UV and 826-UV, with appropriate modifications in view of the small sample volumes. On average replicate analysis of the same sample gave results which did not differ by more than ± 3 %. Blood glucose concentrations were measured in some blood samples taken from the period before and 10 min after the hour's apnoea, using techniques outlined in Sigma Technical Bulletin No. 510.

All results were analysed statistically by Fisher's t-test and 5 % was regarded as the fiducial limit of significance. When mean values are given in the text and figures they are presented ± S. E. M.

Results

a) Oxygen Consumption, before, during, and after One Hour's Apnoea in Air

The average oxygen consumption of the twelve curarised frogs on artificial ventilation before the apnoeic period was 782 ± 18 µl/10 min. During apnoea, which was started with the lungs collapsed, oxygen uptake fell and in the period from 10 to 20 min after onset of apnoea it was significantly below the pre-apnoeic value (Fig. 1). Oxygen uptake stabilized at this value for the remainder of the apnoeic period (Fig. 1).

During the apnoea frogs consumed 983 µl less oxygen than in the hour before and heart rate fell by about 5 beats/min although this change was not significant (Fig. 1).

When artificial ventilation was restarted oxygen uptake rose dramatically and was significantly above that observed in the pre-apnoeic period for the first 10 min of recovery (Fig. 1). During this time frogs consumed 960 ± 50 µl of oxygen, an increase of 178 µl over that consumed in a comparable pre-apnoeic period. The oxygen uptake remained elevated, compared with the pre-apnoeic levels, throughout the remainder of the recovery period of one hour, but the difference was not significant. During the one-hour recovery period the frogs consumed, on average, 350 µl more oxygen than they did in the hour before the apnoea. Heart rate returned to control levels immediately artificial ventilation was restarted.

b) Blood Lactic and Pyruvic Acid Concentrations before, during, and after One Hour's Apnoea in Air

On artificial ventilation before the apnoea average blood lactic acid concentration was 7.74 ± 0.77 mM/l (Table 1), a figure considerably above that reported for Bufonidae (Leivestad, 1960; Armentrout and Rose, 1971), but closer to reported values for Ranidae (Hashimoto and Nukata, 1951). On artificial ventilation the blood glucose level (52.5 ± 3 mg %) was higher than that recorded for *R. tigrina* by Sabnis and Rangnekar (1968) and Maitrya *et al.* (1970) but in the range of values quoted for other Ranidae by Smith (1950, 1954) and Hashimoto and Nukata (1951).

The concentration of lactic acid in the blood increased during the period of one hour's apnoea (Table 1). The highest levels were recorded at the end of the period of apnoea and not during the initial recovery period (Table 1). Blood lactate decreased during the hour following restarting artificial ventilation to 8.79 ± 0.69 mM/l (Table 1). Due to the marked variability in lactic acid levels between animals none of the changes were significant when analysed by Fisher's *t*-test; however as lactic acid concentrations increased in all animals between the pre-apnoeic and end of the apnoeic period, and decreased during the recovery period, the changes in lactate between these periods were significantly different at the 2.5 and 1 % levels respectively when examined by Wilcoxon's paired data test. Blood glucose levels increased by 5.1 mg-% between the pre-apnoeic period and after 10 min recovery from the apnoea (Table 1).

The lactate: pyruvate ratio increased during the apnoea but returned to the pre-apnoeic levels during the recovery period (Table 1). Heart

Table 1. Lactate, pyruvate and glucose concentrations in arterial blood of curarised *R. pipiens* before, during, and after one hour's apnoea in air. Average values from determinations on 15 animals, average weight = 30 ± 2.25 g

	Pre-apnoea	60 min apnoea	10 min recovery	60 min recovery
Lactate (mM/l)	7.74 ± 0.77	9.92 ± 1.04	9.75 ± 1.11	8.79 ± 0.69
Lactate: pyruvate ratio	12.5 ± 2	16.89 ± 2.97	13.19 ± 1.74	11.06 ± 2.09
Heart rate (beats/min)	60 ± 3.5	50 ± 3	55 ± 3	58 ± 3
Glucose (mg-%)	52.2 ± 3	—	57.3 ± 4	—

rate of these animals decreased during the apnoea by 10 beats/min but the rate after 60 min apnoea was not significantly different from that in the pre-apnoeic period. Heart rate rose only when artificial ventilation was restarted and control levels were not attained until after 40 min recovery.

c) Oxygen Consumption before, during, and after One Hour's Apnoea in Oxygen

The average oxygen uptake of the eleven curarised frogs used in this series of experiments was 919 ± 32 µl/10 min in the pre-apnoeic period. However, these animals were significantly heavier than those used in section (a) so that on a unit weight basis the difference in oxygen uptake between the two groups of frogs was negligible. This finding is in agreement with that made previously on *R. pipiens* breathing normally (Jones, 1967); that is, oxygen uptake is not elevated during exposure to pure oxygen. As can be seen from Fig. 2 there were no significant changes in oxygen uptake from the pre-apnoeic level either during the apnoea or the 40 min recovery period.

Discussion

The present data have definitely established that *R. pipiens* can produce energy during apnoea in air by means of anaerobic processes. However, the amount of energy obtained is small compared with that obtained from oxidative metabolism during apnoea. For instance, oxidative processes during apnoea can contribute up to 20.5 calories to the energy budget in this size of frog, assuming that the R.Q. = 1 as was recorded for *R. esculenta* under similar conditions (Jones, 1972b), whereas the total anaerobic contribution is only about 11 % of this value assuming that lactate levels in the muscles are the same as those in blood and that

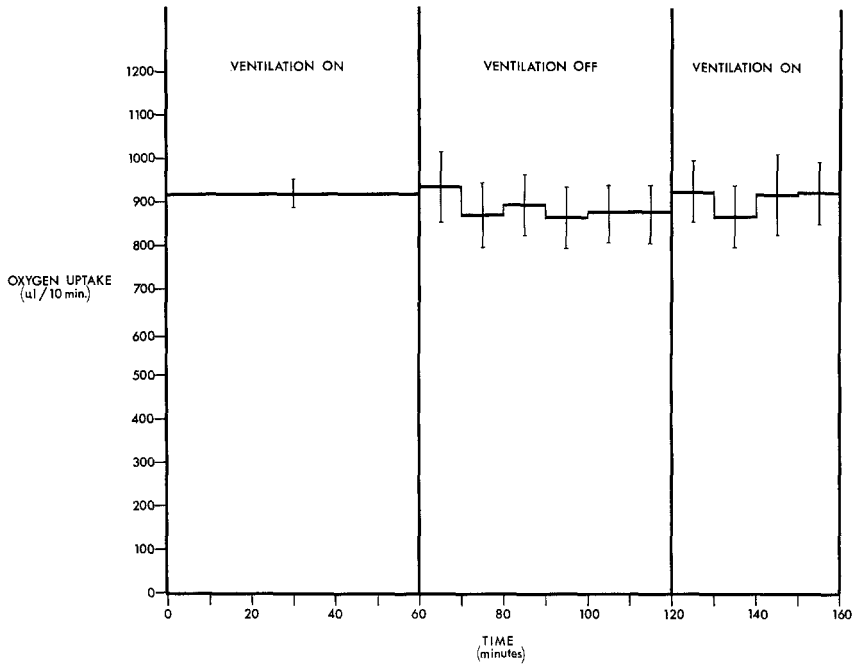

Fig. 2. Oxygen consumption of curarised frogs before, during and after one hour's apnoea in oxygen ($P_{O_2} = 760$ mm Hg). Artificial ventilation was stopped at 60 min and restarted at 120 min. Average values of 11 experiments, average weight = 48.2 ± 3 g ($n = 11$)

these tissues together constitute about 40 % of the animal's total body weight. Huckabee's (1958) basic assumption was that blood lactate and pyruvate concentrations reflect intramuscular concentrations in mammals, although this has not been confirmed in all cases (Karlson, 1971). However, the pattern of change in blood lactacid concentrations during recovery, being quite unlike that seen in higher vertebrates (Scholander, 1940), would suggest that Huckabee's (1958) assumption holds for amphibians under these conditions. The second assumption made in the calculation of the anaerobic contribution to the apnoeic energy budget was that the blood and muscles contributed 40 % of the total body wet weight. Total blood volume is about 8 % of body weight (Prosser and Weinstein, 1950), whereas careful dissection of the muscles from two specimens of *R. pipiens* showed that the muscles contributed about 30–33 % of the body wet weight.

In a survey of oxygen uptake by non-curarised anurans during one hour's submergence at 17° C, it was found that oxygen uptake of *R.*

pipiens (av. wt. = 32 g) was reduced by about one-third from the pre-dive level whereas in *R. esculenta* (av. wt. = 36 g) the reduction was by some 50 % (Jones, 1967). The difference between these two species in regard to cutaneous oxygen uptake has been confirmed by studies on curarised animals during apnoea in air. For curarised *R. esculenta* (av. wt. = 38 g) the reduction in oxygen uptake during one hour's apnoea in air (Jones, 1972b) was more than twice that observed for curarised *R. pipiens* (av. wt. = 37 g). In fact the rather small decrease in oxidative metabolism in the present experiments suggests little reduction in overall energy metabolism during the apnoea, particularly if *R. pipiens* exhibits R. Q. changes akin to those of *R. esculenta* during apnoea (Jones, 1972b). If this is the case then, assuming the same calorific equivalents for fat-carbohydrate metabolism commonly given for man, the pre-apnoeic energy production for *R. pipiens* in section (a) is of the order of 22.9 cals/hr. During apnoea oxidative processes contribute 20.5 cals/hr. to the energy budget and the anaerobic contribution is 2.3 cals/hr [if animals of the weight range used in section (a) undergo lactate changes similar to those in section (b)]. The slight bradycardia shown in the present experiments lends support to the conclusion that the energy budget during apnoea was little changed from that in the pre-apnoeic period (Jones, 1972a). However, this may not obtain for all anurans during periods of solely cutaneous respiration since both Leivestad (1960) and Jones (1972b) recorded decreases in heat production of both toads and frogs during submergence and apnoea in air respectively.

The total oxygen debt displayed by the present animals was about 350 µl in the one hour recovery period. The pattern of oxygen uptake during recovery closely parallels that observed in curarised *R. esculenta* (Jones, 1972b) and can probably be explained on the same basis. That is, the significant increase in uptake over the pre-apnoeic level in the first 10 min of recovery is probably used to replenish the oxygen store of the blood and other more minor stores. Judging from the results of *R. esculenta* (Jones, 1972b) the amount of oxygen required is in the range of 120–130 µl. For *R. pipiens* this would probably be somewhat high since it was shown in *R. esculenta* (Jones, 1972b) that the amount by which arterial oxygen tension falls is directly proportional to the reduction in oxygen uptake and the fall in oxygen uptake in *R. esculenta*, as has already been pointed out, was by 44 % compared with only about half this value in *R. pipiens*. However, it must be appreciated that differences in the shape of the oxygen dissociation curve, the magnitude of the Bohr Effect, the total oxygen capacity of the blood, as well as the degree of cutaneous vascularization, may all contribute to substantial differences between the two species in regard to oxygen uptake during apnoea.

The difference between the oxygen required to replenish stores and the total excess uptake over the recovery period, in the present case some 220–230 μl, probably represents that which is used to repay the lactacid oxygen debt. Huckabee (1958) introduced the concept of "excess lactate", which is calculated from simultaneous lactate and pyruvate concentrations, the assumption being that lactate might be expected, from the concept of the law of mass action, to be formed due to an accumulation of pyruvate, and consequently "excess lactate" would be expected to correlate better with the oxygen deficit. However, recent studies have indicated that, at least during heavy exercise in mammals, lactate accumulation is directly related to the oxygen deficit in exercising muscles (Karlsson and Saltin, 1970). Furthermore, in the present experiments, at the end of the apnoea, blood pyruvic acid levels were slightly below the pre-apnoeic values, a result similar to that in the freshwater turtle after prolonged anaerobiosis (Robin *et al.*, 1964). In view of these results it seems more reasonable to calculate the lactacid oxygen debt from the actual measured values of lactic acid in the blood. Consequently, animals of the weight range used in section (a), if undergoing lactacid changes similar to those in section (b), eliminate 3.365 μM of lactic acid from the blood during the recovery period which, assuming that 20% is oxidized (Scholander, 1940), will require 45 μl of oxygen. Therefore, the total amount of oxygen required if the blood concentrations parallel those of the muscles is five times this value since blood and muscles form 40% of the total body wet weight. When this value is added to the calculated requirement to replenish oxygen stores (120 μl) then the total calculated debt is 345 μl, a value which is in extremely good agreement with the recorded value of 350 μl.

Since the two major components of the post-apnoeic oxygen debt have now been definitely established (Jones, 1972b) it might be predicted that curarised frogs would exhibit no increase in oxygen consumption after one hour's apnoea in oxygen, provided that oxygen metabolism is not changed significantly during the apnoea. This was in fact confirmed in the final series of experiments reported in this paper. However, similar experiments performed previously with non-curarised *R. pipiens* (apnoea being induced by submergence) gave totally different results in that the oxygen debt following submergence in water of $P_{O_2} = 760$ mm Hg was unchanged from that observed after submergence in aerated water ($P_{O_2} = 150$ mm Hg), although oxygen metabolism was not reduced under the former condition (Jones, 1967). In fact the oxygen debt displayed by normal animals during the first 30 min of recovery from submergence in oxygenated water was some 5 to 6 times greater than that recorded in the present animals after one hour's apnoea in air [this calculation being performed on a unit weight basis and after compensation for the

difference in temperature between the two series of experiments (Rieck *et al.*, 1960)]. In view of this the suggestion made, at that time, that the oxygen debt was masked by the oxygen cost of excess activity in the form of hyperventilation appears valid (Jones, 1967). In retrospect it would appear that hyperventilation occuring after no reduction in oxygen metabolism during submergence is caused by the requirement to eliminate carbon dioxide accumulated in blood and tissues (Lenfant and Johannsen, 1967; Jones, 1972b). Consequently it can now be proposed that in normal frogs, during recovery from prolonged submergence, the increase in oxygen uptake above normal levels has three factors: (I) the replenishment of oxygen stores, particularly that of the blood (Jones, 1972b), (II) the repayment of the lactacid oxygen debt (present data) and (III) the provision of metabolic energy for excess activity in the form of hyperventilation (Jones, 1967). The last is by far the major contributor to the elevation in oxygen uptake.

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