Effects of *in vitro* Anoxia on Metabolism of Octopus Heart Tissue.* (27940)

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The cephalopod molluscs are usually thought to be dependent upon a continuous supply of oxygen. The large Pacific coast octopus, however, is capable of withstanding relatively long periods of oxygen deficiency. These animals, if kept cool, may be transported without water for periods up to 2 hours without apparent damage, as evidenced by their normal behavior when returned to sea water. The ability of octopus to discontinue respiratory movements for long periods has been frequently observed during the course of operations on the excretory and circulatory systems.

These observations have led us to investigate the resistance of the tissues of this animal to acute anoxia. Our approach to this problem has been to determine the respiration of tissue slices in an atmosphere of 100% oxygen following exposure to 100% nitrogen. Heart tissue was chosen for the initial experiments because it was thought that this tissue would be quite sensitive to lack of oxygen, as it is in mammals. This paper will report recovery of slices of systemic heart from anoxia.

Methods and procedure. Specimens of *Octopus dofleini* were caught in various areas of Puget Sound and kept in large tanks in the laboratory in running sea water. The temperature in the tanks averaged 10°C, which approximates the environmental temperature for these animals.

Animals were sacrificed by quickly severing the central nervous tissues. The systemic heart was then removed and slices made by hand in a refrigerated, humidified box. The slices were weighed on a Roller-Smith balance and placed in the Warburg vessels. The suspending medium was blood, centrifuged to remove the cellular elements present. In almost all cases, the blood used was taken at the same time, and from the same animal, as the heart tissue. All experiments were run at 13-14°C.

The subsequent procedure was the same in all experiments. Two vessels, to be used as controls, were gassed for 2 minutes with oxygen, and all stopcocks then closed. The remaining vessels were then gassed for 10 minutes with tank nitrogen passed through hot copper screening to remove all traces of oxygen. After 15 minutes equilibration, the control vessels and thermobarometer were set and readings taken for 4-5 hours; for the first hour at 20-minute intervals, and thereafter at 30-minute intervals. After periods of anoxia ranging from 4 to 48 hours, the vessels under nitrogen were gassed with oxygen and the respiration rates of the contained tissues followed for 4-5 hours. Control vessels were reoxygenated at the same time and level of oxygen uptake established.

In the experiments in which glycogen was determined, 6 flasks were set up for each period of anoxia. At the end of the anoxic period, 3 flasks were removed and the pooled tissue from all 3 dropped into 30% KOH for digestion. The remaining flasks were oxygenated and their respiration determined as usual. Glycogen was precipitated in alcohol and then hydrolyzed and determined as glucose by the Anthrone method(5). Readings were made in a Beckman DU spectrophotometer.

Results and discussion. The results of the oxygen uptake determinations are summarized in Table I and Fig. 1. Each value in the Table represents the mean of 13 to 98 determinations. The initial control value represents the mean of determinations on tissue slices during the second and third hours following the start of the experiment. The remaining values in the Table represent the means of the readings taken during the sec-

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TABLE I. Oxygen Uptake of Octopus Heart Slices (microliters/mg/hr × 10) in 100% Oxygen Following Exposure to Complete Anoxia. Values are average of readings taken during 2nd and 3rd hr. Standard errors of means are also given.

		No. of hr in nitrogen			
	Initial	16	24	36	48
Control level	*.105 ± .003	.064 ± .006	.044 ± .002	.039 ± .003	.038 ± .002
Experimental		.068 ± .003	.064 ± .001	.069 ± .008	.031 ± .002

* Mean of 2nd and 3rd hour readings after tissue was placed in flasks.

ond and third hours following oxygenation of both control and experimental vessels, after the experimental vessels had been exposed the indicated number of hours to anoxic conditions.

Preliminary studies showed that 1-7 hours of complete anoxia caused virtually no change in the level of oxygen uptake. Therefore, longer anoxic periods of 16, 24, 36, and 48 hours were chosen for the definitive experiments.

Our results demonstrate a remarkable resistance of octopus heart to anoxia. It is not until the tissues have been left for 48 hours in nitrogen that the respiratory level is markedly depressed. The tissues left in nitrogen for periods up to 36 hours respire, on oxygenation, at higher levels than those left in oxygen. After 16 hours exposure to nitrogen, the level of respiration, on oxygenation, has declined about one-third from initial control level (Fig. 1). However, concurrent measurement of respiration in the control flask indicates that these have decreased by a like amount. After 24 and 36 hours under nitrogen the tissues retain an ability to respire at the same level as after 16 hours, whereas the control flasks continue to decline. After 48 hours the control level has apparently stabilized, but there is a marked drop in recovery oxygen uptake of the anoxic tissues, and many did not respire at all.

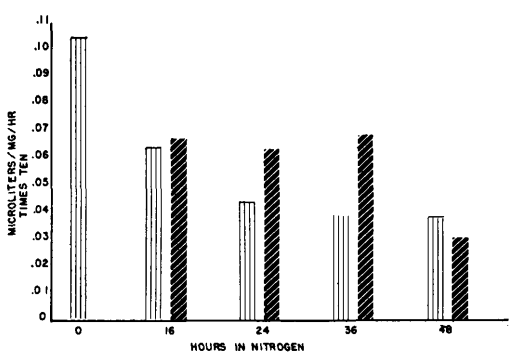

FIG. 1. Oxygen uptake of octopus heart muscle following varying periods of anoxia. Filled bars, level of respiration in experimental vessels during 2nd and 3rd hours following anoxia; open bars, concurrently measured level of respiration in control vessels.

These findings indicate that the octopus heart has a remarkably greater resistance to anoxia than cardiac muscle of mammals. Tissue respiration studies, comparable with the present one, have been done with rats and show that cardiac muscle is extremely sensitive to anoxia. Whether the anoxia is caused by decapitation and subsequent tissue removal(3,4,6) or by exposing the tissue *in vitro* to nitrogen(1,2) respiration is markedly depressed or abolished following 5-60 minutes of anoxic conditions. According to Fuhrman *et al.*(2), 40 minutes of *in vitro* anoxia is sufficient completely to inhibit oxygen uptake of rat heart slices determined subsequently in 100% oxygen. In another paper(3) these authors list several rat tissues in order of increasing resistance to *in vitro* anoxia. Liver and cardiac muscle were the most sensitive, followed by kidney, cerebral cortex and skeletal muscle. No reports have been found of similar *in vitro* anoxic studies on the heart tissue of any cold-blooded animal.

Recently, Meyer and Purdy(7) have shown that there is an 80% decrease in both free and bound cardiac glycogen in rats following only 9 minutes of acute anoxia. Cordier(1) reports a several hundred fold decrease in cardiac glycogen following fulminating anoxia. To determine whether glycogen was being utilized by octopus heart under conditions of complete anoxia, the tissue contents of some of the reaction vessels which had been exposed to nitrogen were removed

for glycogen analyses. With the exception of Experiment 11, the results (Table II) indicate considerable glycogen depletion. Exp. 11 shows less percentage decrease than the others, but this may be the result of the much lower initial glycogen level in the heart of this animal. In 2 experiments, glycogen was analyzed in tissues taken from control vessels after respiring in air for 48 hours. The results (Table III) show a depletion of heart muscle glycogen under these conditions, but to a much lesser extent than in the tissues exposed to anoxia (Table II).

If glycolysis were proceeding actively in the anoxic tissues, it would be expected that acid products would accumulate. This was roughly tested by measuring pH of the suspending medium at termination of the experiment, at which time the tissues had been respiring in 100% oxygen for at least 5 hours following reoxygenation of the vessels. The average pH in vessels exposed to 24 hours of anoxia was 6.94; in vessels exposed to 48 hours anoxia, 6.42; compared to an average of 7.20 in the control vessels. Values of pH were also obtained from vessels removed for glycogen analysis, thus giving values representing conditions immediately following anoxia. After 24 hours anoxia, the pH had fallen to 6.53; after 48 hours to 6.63.

It appears, therefore, that octopus heart muscle utilizes glycolysis as at least one source of energy during anoxia. In an animal like the octopus the heart stops beating during anoxia and although the carbohydrate reserves are small, rate of metabolism is very low within the normal temperature range. Thus, the carbohydrate reserve may be sufficient for the duration of anoxia likely to be encountered, for example, through a tidal period. It must be emphasized, however, that in this study only a preliminary approach has been made to an understanding of the metabolic processes in octopus heart muscle.

TABLE II. Glycogen in Octopus Heart Muscle Before and After *in vitro* Exposure to Complete Anoxia. Values in the table are mg glycogen (as glucose)/g of tissue. Each value, except initial glycogen samples, represents a combined tissue sample from 3 reaction vessels.

Exp No.	Initial glycogen (mg/g)	Period of anoxia (hr)	Glycogen immediately following anoxia (mg/g)	% decrease
11	5.8	16	3.2	45
12	10.8	16	1.3	88
		24	1.0	90
14	10.0	36	3.0	70
		48	3.3	67
15	12.8	36	5.2	60
		48	5.4	58

TABLE III. Glycogen in Tissue from Control Vessels After 48 hr Respiring in Air and 100% Oxygen. Values in mg glycogen (as glucose)/g of tissue.

Exp No.	Initial glycogen (mg/g)	Final glycogen (mg/g)	% decrease
14	10.0	6.3	37
15	12.8	7.5	41

Summary. 1. The oxygen uptake of octopus heart tissue slices was determined following 16, 24, 36 and 48 hour exposures to *in vitro* anoxia. Only after 48 hours exposure was recovery oxygen uptake depressed below the control level. 2. Glycogen measurements on the tissue from the reaction vessels indicated a 60-90% decrease in glycogen following *in vitro* anoxia. There was no relationship between length of anoxia and percentage decrease in glycogen. 3. Values of pH in the suspending media of the experimental vessels, taken at the end of experiment, were considerably below those of the controls. 4. It was concluded that octopus heart muscle is much more resistant to anoxia than the cardiac muscle of higher animals.

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