THE ROLE OF TISSUES IN THE ANAEROBIC METABOLISM OF THE MUSSEL ANODONTA HALLENBECKII LEA

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Introduction

Lamellibranch mollusks possess the capacity for enduring anaerobiosis for a considerable time. Such a capacity is advantageous to tidal zone forms which are exposed to air at low tide, and likewise to freshwater mollusks which may have to endure low oxygen content of polluted water as well as exposure. Recognition of this peculiar ability has led to investigation of anaerobic metabolic processes of mussels. If the stream of water passing over the gills is cut off, the oxygen supply fails while carbon dioxide accumulates. The manner in which the mollusk deals with accumulating carbon dioxide has been the subject of several investigations.

Collip (1921) showed that the marine form *Mya arenaria* used calcium to buffer carbon dioxide. Dotterweich and Ellsner (1935) showed that in the freshwater mussel *Anodonta cygnea* most of the carbon dioxide formed during anaerobiosis entered into combination with calcium to form calcium bicarbonate. A small amount was buffered by calcium proteinate. They concluded that in general calcium in the shell of mollusks may be utilized as an alkali reserve.

Recent investigation by Dugal and Irving (1937) indicated that tissues as well as body fluids are involved in adjustment to oxygen lack. Mantle tissue of *Venus mercenaria* was found to accumulate carbon dioxide and calcium just as did mantle cavity fluid.

The work reported in the present paper was an investigation of the adjustment of a freshwater mollusk to a disturbance of the acid-base balance resulting from anaerobiosis. Particular reference was made to the rôle of mantle and gill tissues in this adjustment. Determinations of the carbon dioxide content gave results which indicated that mussel tissues were able to buffer carbon dioxide. The relation of calcium to the buffering process was studied. Observations were made on the oxygen consumption of tissues taken from asphyxiated animals. Evidence of an oxygen debt was found, showing that dissimilative processes were continuing through the period of anaerobiosis.

MATERIALS AND METHODS

Animals used were freshwater mussels taken in the vicinity of Durham, N. C. They were identified by Dr. Henry van der Schalie of the University of Michigan Museum of Zoölogy as *Anodonta hallenbeckii* Lea.

Control animals were kept in tanks of running water. In this situation the valves remained open, allowing a constant stream of water to pass over the gills. Experimental animals were removed from such tanks and placed in a refrigerator with an air temperature of 6 to 8° C. At this temperature clams survived about a month. When removed from water *Anodonta* closed the shell valves. In this position exchange of gases between animal and environment was impossible. Any opening of the shell was accompanied by leakage of fluids from the mantle cavity. Leaking animals were not included in the experiments.

Tissues used were gill, mantle, and kidney tissue. Some observations were made on pallial muscle and foot muscle.

The rate of oxygen consumption of gill, mantle, and kidney tissue was measured in a standard Warburg apparatus. Tissue samples weighing about 0.1 gram were suspended in a salt solution containing 0.153 per cent NaCl. Absorption of carbon dioxide was accomplished with 20 per cent KOH. The temperature was held at 25° C. Measurements were made over a period of sixty minutes.

Carbon dioxide content of gill and mantle was determined by an adaptation of the Van Slyke manometric method for the determination of blood gases. The gas burette of the apparatus was modified from that described by Ferguson and Irving (1929). A ground joint at the lower end of the extraction chamber allowed the introduction of tissue. A weighed sample of tissue was placed in the extraction chamber, the burette put in place, and the joint made secure. Carbon dioxide was liberated by 0.1 N HCl introduced through the upper stopcock. Usually complete extraction required 45 minutes of shaking. Carbon dioxide was absorbed with air-free 1.5 N NaOH. The values P_1 and P_2 and the correction factor, *c*, were determined in the usual way.

Conversion of the observed pressure of carbon dioxide into cubic centimeters of gas was made according to the formulae modified for use with tissue samples by Ferguson and Irving (1929). Values for specific gravity were necessary for the conversion formulae. These values as determined were: for mantle, 1.04; for gill, 1.12.

Care was taken to maintain constancy in the method of obtaining and weighing tissue samples. It is felt that the values for carbon dioxide content are comparable, although they may not be absolute.

Calcium content of clam tissues was determined from samples digested in a mixture of concentrated nitric and perchloric acids. Calcium was precipitated from the digest as oxalate, redissolved and titrated with permanganate.

Results

Oxygen Consumption

Respiration of tissues from aerobic and anaerobic animals was compared. The results are given in Table I. The values for control animals are based on four determinations. They agreed closely. There were wider differences in the determinations on tissues from anaerobic animals. These values have been arranged by length of anaerobic period, and also averaged into one value for asphyxiated animals.

Table I

Oxygen uptake of gill, mantle, and kidney tissue. Values are averages of two to four determinations and represent cubic millimeters of oxygen consumed per hour per milligram dry weight of tissue at 25° C.

Days out of water	Kidney	Mantle	Gill
14	4.70	1.32	.486
10	3.66	1.34	.766
8	2.69	1.24	.652
6	2.82	1.45	.758
4	3.24	1.02	.660
2	2.82		.573
Average	3.32	1.27	.649
Average of controls	2.11	1.02	.421

Tissues removed from asphyxiated animals consumed more oxygen per hour per unit weight than did tissues from control animals. This was true for the first hour after removal. Determinations were not carried beyond this point. It is therefore impossible to make any calculation of the total extra oxygen required. However, the increase noted suggests the paying off of an oxygen debt incurred during anaerobiosis.

The respiration rates are referred to dry weight of tissue. It was found that mussel tissues varied in water content from one individual to another. There was no evidence of a correlation between dry weight and anaerobic period. The observed percentages dry weight as averaged from a large number of samples studied are given below:

mantle	3.9
kidney	8.6
gill	24.0

It is interesting that the rate of oxygen consumption of kidney tissue was much higher than that of other tissues studied. According to Holmes (1937), the high rate of respiration of mammalian kidney tissue is due to osmotic work done by excretory cells. Probably a similar explanation fits the case of mussel kidney.

Carbon Dioxide Content

Results of the determination of the carbon dioxide content of gill and mantle are given in Table II. The following points are to be noted:

TABLE II

Carbon dioxide content of mantle and gill. Values are expressed as cubic centimeters of gas at standard temperature and pressure and equivalents of carbon dioxide in one hundred grams fresh tissue. Averages of several determinations are represented.

Days out of water	Mantle	Gill	Mantle	Gill
	cc./100 gr.	*cc./100 gr.*	*equiv./100 gr.*	*equiv./100 gr.*
0	25.0	322	0.0022	0.0287
1		399		0.0356
2	32.2	372	0.0028	0.0332
3	35.0	369	0.0030	0.0328
4	32.0	405	0.0028	0.0376
6	34.4	430	0.0030	0.0392
8	43.6	455	0.0038	0.0406
10	44.2	487	0.0038	0.0432
12		499		0.0444
14	47.7	512	0.0042	0.0456

1. Gills contained approximately ten times as much carbon dioxide as did mantles.

2. There was a steady increase in the amount of carbon dioxide accumulating in gill tissue during anaerobiosis.

3. Carbon dioxide accumulated in mantle tissue in proportion to the increase in gill tissue. The equivalents of carbon dioxide in mantle doubled during asphyxiation.

For purposes of comparison with the amount of calcium present, the values for carbon dioxide were converted into equivalents and are also given in Table II.

Calcium Content

It was found that the calcium content of the tissues studied did not vary significantly with the period of anaerobiosis. Averages from a large number of determinations are given below, expressed as milligrams of calcium per gram dry weight of tissue.

foot muscle	8	mg./gram tissue
pallial muscle	31	"
kidney	46	"
mantle	62	"
gill	175	"

Gill tissue contained a large amount of calcium as compared with other tissues. This may be correlated with the relatively high dry weight of gill tissue. The small amount of calcium found in foot muscle is surprising when considered with the other values.

By using the percentage dry weight of mantle and gill tissue it was possible to calculate equivalents of calcium per one hundred grams fresh tissue. These were found to be: for mantle, 0.0045; for gill, 0.21.

Discussion

Study of the functioning of animal tissue in buffering processes has not been investigated in many species. Dotterweich (1933) showed that the calciferous glands of earthworms were capable of giving up calcium to buffer carbon dioxide accumulating in body fluids. Banus and Katz (1927) found weak buffering by hind leg muscles of a cat. A similar effect was noted by Irving and Chute, (1932) in muscle.

A buffer system in the tissues of *Anodonta* is indicated by a study of the carbon dioxide and calcium content of certain tissues. Gill tissue seems to be most active in this respect.

From the data given above, it is seen that one hundred grams fresh gill tissue contain 0.21 equivalents of calcium, and 0.0287 equivalents of carbon dioxide (see Table II). This proportion indicates that most, possibly all, the calcium is present in some form other than carbonate.

During anaerobiosis the carbon dioxide level rises, increasing to 0.0456 equivalents at 14 days. This increase is not accompanied by an increase in the hydrogen ion concentration. The hydrogen ion concentration of the tissue was measured colorimetrically, and was found to vary less than 0.05 from pH 6.8 for gill, 6.9 for mantle. Apparently the accumulating carbon dioxide is bound in some way so that an increase in hydrogen ions does not occur.

It was suggested by Dotterweich and Ellsner (1935) that a calcium-proteinate might act as an additional buffer in the fluid of *Anodonta cygnea*. In that system calcium carbonate was the principal alkali reserve. In the tissues of *Anodonta hallenbeckii* it would seem that calcium-proteinate, or some other combination of a weak acid with calcium, is the chief buffer, with the carbonate playing at the most a minor rôle.

In the case of mantle tissue 0.0045 equivalents of calcium are present in the normal mantle. Carbon dioxide increases from 0.0022 equivalents in the normal tissue to 0.0042 equivalents in the asphyxiated tissue. The calcium and carbon dioxide are then in a one-to-one ratio. This would indicate a more limited calcium reserve in mantle than in gill.

Dugal (1939) has shown that in *Venus* the calcium reserve may be augmented by calcium from the shell. Tissues of *Anodonta* maintain a steady calcium level.

Calcium is not only the chief component of the hard parts of mollusks but also forms a considerable portion of the alkali reserve. The same factors which govern the precipitation of solid calcium in the shell are responsible for the deposition of calcium in tissues. It is a point of interest that freshwater clams possess large deposits of calcium in their gills, and marine clams possess the larger deposits in mantle tissues (McCance and Shipp, 1933). There may be some correlation here with the fact that glochidia develop in the gill pouches of freshwater mussels and may derive calcium for their shells from the abundant supply available.

Jatzenko (1928) showed that certain freshwater mussels build up an oxygen debt during anaerobiosis. It is to be expected that individual tissues would also show such a debt. All activity does not cease when the clam is temporarily asphyxiated. Some of it continues. Ciliary action such as accounts for a great deal of the oxygen consumption of gill and mantle probably does decrease to some extent. Osmotic work which is characteristic of kidney tissue continues and may even increase during anaerobiosis. Data for individual tissues as presented in Table I show that oxygen consumption of mussel tissues is higher immediately after a period of asphyxiation than under normal conditions.

The source of energy for activities carried on during anaerobic periods is generally laid to a glycolytic process. However, there has as yet been no isolation of the tissue or tissues mainly responsible for the glycogen reserve. The problem of the energy source and its localization is a pertinent one to a complete explanation of the anaerobic metabolism of mussels.

Summary

Tissues of *Anodonta hallenbeckii* are capable of buffering carbon dioxide accumulating during anaerobiosis. Calcium compounds present in gill and mantle serve as an alkali reserve. During anaerobiosis carbon dioxide increased in the tissues studied while the hydrogen ion concentration remained constant. It is concluded that accumulated carbon dioxide was buffered by calcium present.

Gills contain large amounts of calcium which is present in some form other than carbonate.

Kidney tissue showed a very high rate of respiration. Mantle and gill showed low rates. After anaerobic periods the rate of respiration showed a tendency to increase. This may be taken as evidence that these tissues continued to do work during anaerobiosis.

LITERATURE CITED

Banus, M. G., and L. N. Katz, 1927. Observations of the role of tissues in maintaining the acid-base equilibrium of the blood. *Am. Jour. Physiol.*, **81**: 628–649.

Collip, J. B., 1921. A further study of the respiratory processes in Mya arenaria and other marine molluscs. *Jour. Biol. Chem.*, **49**: 297–310.

Dotterweich, H., 1933. Die Function tierisches Kalkablagerungen als Pufferreserve im Dienste der Reaktionsregulation. *Arch. f. die ges.-Physiol.*, **232**: 263–286.

Dotterweich, H., and E. Ellsner, 1935. Die Mobilisierung des Schalenkalkes für die Reakstionsregulation der Muscheln (Anodonta cygnea). *Biol. Zbl.*, **55**: 138–163.

Dubuisson, M., and Y. Van Heuverswyn, 1931. Recherches histologiques et chimiques sur les branchies d'Anodonta cygnea. *Arch. de Biol.*, **41**: 37–74.

Dugal, L.-P., 1939. The use of calcareous shell to buffer the product of anaerobic glycolysis in Venus mercenaria. *Jour. Cell. and Comp. Physiol.*, **13**: 235–251.

Dugal, L.-P. and L. Irving, 1937. Sécrétion de carbonate de calcium par les Venus mercenaria fermées hermétiquement. *Compt. Rend Soc. Biol.*, **124**: 526–528.

Ferguson, J. K. W., and L. Irving, 1929. A method to determine the CO_2 content of muscle. *Jour. Biol. Chem.*, **84**: 143–153.

Holmes, Eric, 1937. The metabolism of living tissues. Cambridge University Press, pp. x–235.

Irving, L., and A. L. Chute, 1932. The participation of the carbonates of bone in the neutralization of ingested acid. *Jour. Cell. and Comp. Physiol.*, **2**: 157–176.

Jatzenko, A. T., 1928. Die Bedeutung der Mantelhöhlenflüssigkeit in der Biologie der Süsswasserlamellibranchier. *Biol. Zbl.*, **48**: 1–25.

McCance, R. A., and H. L. Shipp, 1933. The magnesium and other inorganic constituents of some marine invertebrates. *Jour. Mar. Biol. Ass. Plymouth*, **19**: 293–296.