THE RESPIRATORY DILUTION EFFECT IN SEA-URCHIN SPERMATOZOA

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INTRODUCTION

The Respiratory Dilution Effect is defined as the increase in O_2 uptake, per unit number of sea-urchin spermatozoa, in dilute as compared with dense suspensions, when O_2 uptake is measured manometrically with absorption of CO_2. A dense suspension is one in which semen is diluted 1 : 30 with sea water (app. 7×10^8 spermatozoa/ml); a dilute suspension is one in which semen is diluted 1 : 90 with sea water (app. 2×10^8 spermatozoa/ml).

The respiration of sea-urchin spermatozoa is sensitive to changes in the pH of the suspending medium (Rothschild, 1956). When, for example, the pH is reduced from its normal value of 8.1 to 7.6, there is a sharp fall in O_2 uptake. As sea water is poorly buffered in comparison with the solutions normally used in manometric work, its use as the suspending medium in respirometric experiments makes the results extremely difficult to interpret. This applies both to the Direct and Indirect methods of measuring O_2 uptake, because in the former, the pH continually rises during the experiment, while in the latter, the pH falls to unphysiological values. These considerations raise a number of new problems, of which the following are dealt with in this paper :

(1) What are the quantitative effects of changes in the pH of the medium on the respiration of sea-urchin spermatozoa ?

(2) Can the pH of sea water be sufficiently controlled, by the addition of buffers, to make investigation of the Respiratory Dilution Effect meaningful ?

(3) If questions (1) and (2) can be satisfactorily resolved, what metabolic differences are there between dense and dilute suspensions of sea-urchin spermatozoa ?

As regards question (3), the experiments of ROTHSCHILD (1948) and MOHRI (1956 *a*, *b*) indicate that the cytochrome system of sea-urchin spermatozoa is more sensitive to inhibitors when the spermatozoa are dilute than when they are dense. The experiments of TYLER & ROTHSCHILD (1951) and ROTHSCHILD & TYLER (1954) suggest that trace metals in normal sea water are responsible for these metabolic differences.

A convenient buffer with which to control the pH of sea water is glycyl glycine; sea water containing this compound is referred to as gly.gly.-sea water. Glycyl glycine, however, chelates heavy metals and it follows that the use of gly.gly.-sea water, however desirable from the point of view of pH control, suffers from the disadvantage of making the trace metal hypothesis unverifiable. Boric acid is an alternative buffer and some experiments in which borate-sea water was used as the suspending medium are described in this paper. But when the concentration of borate is adequate from the point of view of pH control, the viability of the spermatozoa is impaired.

MATERIAL AND METHODS

Semen of *Paracentrotus lividus* (Lamarck) and of *Echinus esculentus* Linn. Differential manometers of the Warburg type; vol. 17-21 ml; $kO_2 = 1.5$; 95 c/m; 10 % KOH and filter papers in centre cup. T°C, 15. Air in gas phase. O_2 saturation experimentally found to be adequate.

RESULTS AND DISCUSSION

The effect of semen on the pH of the medium. Table 1 shows the change in the pH of gly.gly.-sea water on the addition of semen (1 : 30), and after incubation for 60 min. in a manometer flask in which the evolved CO_2 was absorbed, the gly.gly.-sea water having been brought to a particular pH before the start of the experiment by addition of isotonic NaOH in distilled water. The relatively low pH of semen, 7.40, the evolution of respiratory CO_2 when it is diluted, and the subsequent absorption of the CO_2 by alkali in the manometer flask make it impossible to « lock » the pH of the suspension except at a concentration of glycyl glycine which is biologically undesirable. If the buffer

TABLE I

CHANGES IN THE pH OF GLY. GLY.-SEA WATER BEFORE AND AFTER A MANOMETRIC EXPERIMENT WITH ABSORPTION OF CO_2

Molarity of glycyl glycine	Before addition of semen	Immediately after addition of semen	After 60 mins. incubation in manometer
0.05	7.49	7.41	7.59
	7.75	7.62	7.78
	8.04	7.86	7.96
	8.30	8.18	8.16
	8.63	8.47	8.30
	8.98	8.75	8.48
0.09	7.66	7.56	7.77
	7.79	7.71	7.88
	8.04	7.90	7.95
	8.28	8.15	8.15
	8.50	8.37	8.33
	8.78	8.63	8.55
	9.06	8.79	8.72
0.21	7.65	7.57	7.66
	7.80	7.70	7.80
	8.00	7.90	7.95
	8.25	8.16	8.16
	8.50	8.41	8.38
	8.75	8.65	8.56
	9.00	8.83	8.76

capacities of three different concentrations of glycyl glycine are compared, Fig. 1, it is evident that a good deal would be gained by using 0.21 M gly.gly.-sea water, but little by using 0.09 M instead of 0.05 M gly.gly.-sea water. Bearing in mind that the latter has a far better buffer capacity than sea water (ROTHSCHILD, 1956, Fig. 2), it seemed best to use the lowest of the three glycyl glycine concentrations.

Fig. 2 shows the variation with pH in the O_2 uptake of a sperm suspension prepared by diluting semen 1 : 31 (7.74×10^8/ml) with gly.gly.-sea water, 0.05 M. The pH associated with each point on the graph is the average of the pH of the suspension at the beginning and end of the experiment; the horizontal lines indicate the difference between each pair of these pH values, the higher, pH value being at the end of the experiment. The most interesting part of the graph is that between pH 7.91 and 8.17. Although an increase of 0.26 pH units might be considered small, it has a profound effect on the respi-

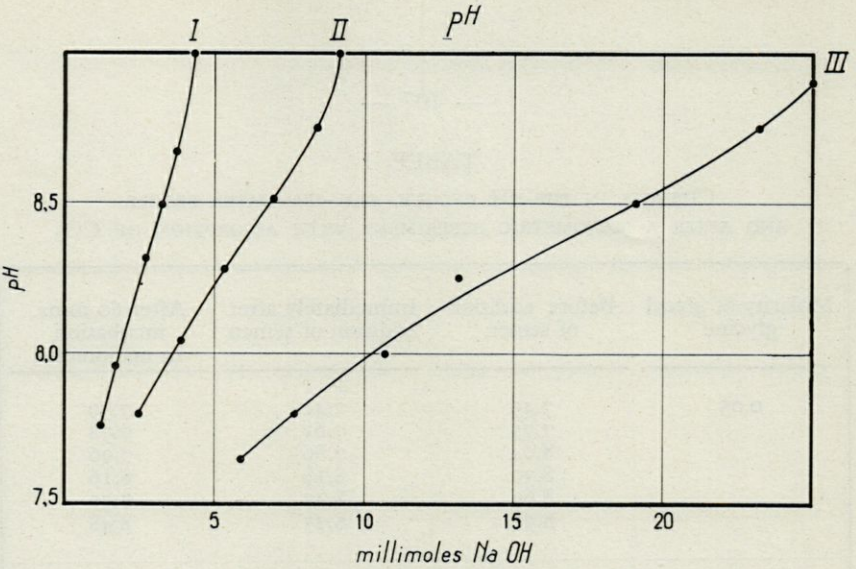

Fig. 1. — Titration of sea water containing 0.05 (Curve 1), 0.10 (Curve II) and 0.30 (Curve III) molar glycyl glycine.

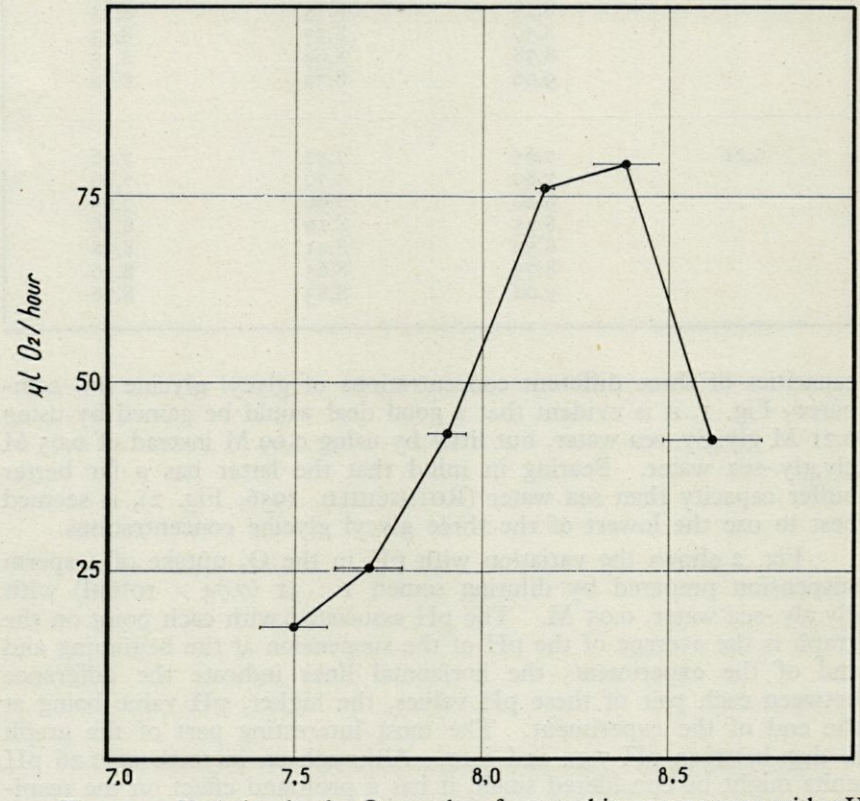

Fig. 2. — Variation in the O_2 uptake of sea-urchin spermatozoa with pH of suspending medium, gly.gly.-sea water, 0.05 M. For further details, see text.

ration of the spermatozoa; this change in pH causes an almost two-fold increase in O_2 uptake. In other experiments using 0.09 M gly.gly.-sea water, an increase in pH from 7.84 to 8.00, 0.16 pH units, increased the O_2 uptake of the sperm suspension by 400 %. These experiments make clear how difficult it is to be certain that the Respiratory Dilution Effect exists, even when questions of O_2 saturation have been satisfactorily disposed of. The addition of ordinary sea water to a suspension, either before the beginning of an experiment, or from side-arms during an experiment, is almost impossible to achieve without changing the pH of the suspension. A difference of 0.2 pH units between the diluent and the suspension to be diluted may cause a 100 % increase in the O_2 uptake of the suspension, at any rate temporarily. In the past this would have been interpreted as an interesting Respiratory Dilution Effect.

Inhibition of the cytochrome system. ROTHSCHILD (1948) and MOHRI (1956 *a*, *b*) reported that spermatozoa in dilute suspensions were more sensitive to cytochrome inhibitors than those in dense suspensions. Table 2 shows that a difference in sensitivity to CO in the dark is

TABLE II

INHIBITION OF SEA-URCHIN SPERM RESPIRATION BY CO IN THE DARK, AT DIFFERENT pH'S. SUSPENDING MEDIUM, GLY. GLY.-SEA WATER 0.05 M. SPERM DENSITY, 7×10^8/ML.

pH	O_2 uptake in μlo_2/hr./ml.		% inhibition
	in 10 % O_2/90 % N_2	in 10 % O_2/90 % CO	
8.30	67	16	76
7.67	9	7	22

observed, *at constant sperm density*, when spermatozoa are induced to respire at different rates by varying the pH. One interpretation of this experiment is that when, for whatever reason, sea-urchin spermatozoa are respiring sub-maximally, the inhibitor acts partly on that fraction of the cytochrome system which is unsaturated by substrate-dehydrogenase complexes. The percentage inhibition is, therefore, less than when the cytochrome system is saturated. During sub-maximal respiration, the action of the inhibitor might therefore be likened to the application of a brake to a « fifth wheel in the coach ».

Table 3 gives the results of an experiment in which borate-sea water was the suspending medium and a Respiratory Dilution Effect was observed. This might be interpreted as showing that the lack of Respiratory Dilution Effect in gly.gly.-sea water is due to the chelating effect of glycyl glycine. This conclusion is, however, unjustified in the

TABLE 3

RESPIRATION OF SEA-URCHIN SPERMATOZOA IN BORATE-SEA WATER, 0.05 M, pH 8.30

Sperm density	$-\mu lo_2/hr./7 \times 10^8$	pH at end
7×10^8	22	8.31
3.5×10^8	33	8.30
2.3×10^8	71	8.31

absence of information regarding the changes with time in the pH of the suspension, even though its pH was 8.3 at the beginning and end of the experiment. As 0.05 M borate-sea water is less well buffered than 0.05 M gly.gly.-sea water, the fall in pH on addition of semen will be greater in borate- than gly.gly.-sea water. This fall will not be reversed immediately and while the pH of the suspension is sub-normal, sperm respiration will be depressed. The effect will not be so marked in dilute suspensions in which the fall in pH after addition of semen will be less pronounced.

TABLE 4

RESPIRATION OF SEA-URCHIN SPERMATOZOA IN BORATE-SEA WATER, 0.05 M, $+ 10^{-3}$ M versene, pH 8.30

Sperm density	$-\mu lo_2/hr./7 \times 10^8$	Notes
7×10^8	11	Not diluted
3.3×10^8	26	Diluted before exp.
3.3×10^8	26	Diluted during exp.

Unless the trace metal hypothesis is abandoned, there may be a similar explanation of the experiment whose details are given in Table 4, as a Respiratory Dilution Effect apparently took place in the presence of a high concentration of versene.

CONCLUSION

The dramatic effects of pH changes on the O_2 uptake of sea-urchin spermatozoa make it clear that sea water buffered with glycyl glycine or some non-chelating buffer (if one can be found), must be used in manometric and, probably, metabolic experiments on sea-urchin spermatozoa. If an adequate non-chelating buffer cannot be found, the only way to establish with certainty that versene inhibits the « Respiratory Dilution Effect » would be by simultaneously doing the following manometric experiments : (1) A calibration experiment to determine the relationship between pH and O_2 uptake in the actual sperm suspension in use, with borate (0.05 M) – versene (10^{-3} M) – sea water as the suspending medium. (2) A series of controls (see ROTHSCHILD, 1956, Fig. 4) to determine the time course of the pH changes in the suspensions in the manometers. (3) Dilution of aliquots of the same suspension by addition of borate-versene-sea water from side-arms. Similar experiments will have to be done to establish with certainty the existence of other reported metabolic differences between dense and dilute sea-urchin sperm suspensions and, for example, the effects of albumin (WICKLUND, 1949, 1954) on the O_2 uptake of sea-urchin spermatozoa.

The hypothesis that dilution of a sea-urchin sperm suspension induces the activation of the cytochrome system, independently of pH changes in the suspending medium, requires revision.

SUMMARY

1. The pH dependence of the O_2 uptake of sea-urchin spermatozoa has been quantitatively examined. A change of 0.26 pH units may cause a 100 % change in the rate of O_2 uptake.

2. The hypothesis that the Respiratory Dilution Effect is specifically associated with the activation of the cytochrome system has been investigated. When the O_2 uptake of sea-urchin spermatozoa is increased or decreased by treatments other than dilution, e.g. by changing the pH, there is a corresponding increase or decrease in the inhibition of O_2 uptake by inhibitors of the cytochrome system.

3. Experiments are described in which a Respiratory Dilution Effect was observed when the suspending medium was 0.05 M borate-sea water, with and without 10^{-3} M versene. The difficulty of interpreting such experiments because of pH changes in the suspension are discussed.

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