A NOTE ON THE RESPIRATION OF ARBACIA EGGS*

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Tang (2), with Gerard (4), measured the respiration of resting and fertilized eggs of *Arbacia punctulata* incidental to a study of the influence of oxygen tension on respiration. Absolute values in cubic millimeters of oxygen per million eggs per hour were reported. For one season the average for unfertilized eggs was 33.6 at 24.7°C., and a few runs with fertilized eggs gave figures five times as high. Next season the average for freshly fertilized eggs was 118 at 25°C., and a few experiments with unfertilized ones gave again one-fifth the respiration, or 23.5. The eggs of the first season, however, averaged 77 micra in diameter and possessed, therefore, almost 25 per cent more volume than those of the second season, with a diameter of 72 micra on the average. Expressed per unit volume of eggs, therefore, the data for the two seasons' experiments are less than 15 per cent apart.

Whitaker (5) has measured again the respiration of this egg and obtained absolute values per unit volume only one-third to one-half as great as those we reported. His results fitted data for the respiration of other related or unrelated eggs, obtained by himself and others, better than did ours, and our procedures and findings were adversely criticized (6). It seemed desirable to clear up, if possible, the discrepancy and establish the correct value; the more so since this datum is becoming of some theoretical importance for recent respiration studies (*e.g.* (1) and (6)).

We have therefore repeated the measurements with careful control of all points of difference in the conditions of our respective experiments, though most of these had been previously considered. Since

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all agree that fertilization gives about a fivefold increase in respiration, and the findings on resting eggs have been especially questioned, the present experiments have been limited to the latter.

There are four major differences to be considered: preparation of the egg suspension; temperature used; conditions during the run in the manometers; measurement of amount of material used.

Our preparation involved: opening the female urchins, placing the ovaries in sea water, straining the thick suspension of eggs through cheese-cloth, and using as such or after two washings with sea water by gentle hand centrifuging. Whitaker allowed eggs to shed through the gonopores of inverted half shells and washed by decantation only. He believed our eggs suffered cortical damage during preparation, with consequent abnormally high respiration. In the present work, we performed two series of tests in which alternate urchins in succession were handled by one or the other method, the two groups collected, and respiration measured. There was no difference. Dr. A. J. Goldforb has kindly permitted us to mention unpublished experiments of similar import. Fertilization, cytological changes, development, and permeability of eggs shed by one female through the gonopores was the same as that of eggs shed from an isolated ovary of the same animal. Likewise he found filtration through cheese-cloth without effect.[1] We have none the less used Whitaker's procedure in the present work. In a few cases, only the Aristotle's lantern was removed and the eggs allowed to shed with a minimum of contamination by body fluids or débris. Further, filtered sea water was used for collecting and washing the shed eggs.

Our experiments were performed at 24.7° or 25°C., Whitaker's at 21°C. For comparing values he corrected our results to 21°, assuming a temperature coefficient of 2.0. As reported in a paper soon to be published, this value is approximately correct only for fertilized eggs, that of resting eggs is over 4. The temperature correction Whitaker applied was, therefore, too low. We shall return to this later.

The most serious discrepancy, Whitaker believed, resulted from the treatment in the manometers for measuring oxygen consumption. Since the chambers containing the respiring egg suspension have liquid and gas phases and oxygen diffusion is slow, continued agitation

[1] Goldforb has also observed a progressive increase in the respiration of unfertilized eggs on standing, beginning some 5 hours after shedding. Our runs have mostly terminated about this time, but we have noted in many longer runs a slight tendency to increased respiration at the end. We would also like to note, for later comparison, that he was not able to obtain constant volume determinations by centrifuging, nor were we.

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is needed to maintain the oxygen content of the liquid. This is achieved by a rhythmic to-and-fro motion of the manometer and, if inadequate, the measured respiration will be less than the true value. The other danger is that this shaking, if too vigorous, will damage the delicate egg cortex, even to the extent of cytolysis, and so produce artificially high oxygen consumptions. It is into this latter error, Whitaker concludes, that we fell. Though our actual shaking conditions were specified only for the fertilized eggs, the

TABLE I

1 cc. unfertilized *Arbacia* eggs (477,000). 0.2 cc. N NaOH in inset. 21°C. Start = 94 per cent fertilization, end = 95 per cent fertilization. Manometer constants all about 0.8.

Shaking	Time	Change in level			
		1	2	3	4
	min.	*mm.*	*mm.*	*mm.*	*mm.*
48/min., 5.5 cm. arc	0–20	2.3	2.7	2.5	2.7
	20–40	2.2	2.2	2.2	2.1
	40–60	2.6	2.3	2.3	2.8
Q_{O_2} 1 hr.		**12.1**	**12.8**	**13.6**	**13.0**
	60–120	7.6	7.6	6.9	7.7
Q_{O_2} 2 hrs.		**12.7**	**13.0**	**13.3**	**13.2**
72/min., 5.5 cm.	120–150	4.1	3.6	3.5	3.6
	150–180	4.2	4.0	4.0	4.4
Q_{O_2} 1 hr.		**14.2**	**13.4**	**14.6**	**13.8**
	180–300	14.9	14.2	13.3	14.3
Q_{O_2} 3 hrs.		**14.3**	**14.0**	**14.7**	**14.0**

questions of oxygen penetration and egg injury were fully considered. Our fluid layer was less than 2 mm. deep, Whitaker's 3.5 or 7, and the required agitation is, of course, much less with thinner layers. In fact, in the unfertilized egg experiments, shaking rates varied from 40 to 60 per minute over arcs of 5 to 10 cm. with no regular differences in results. After a run, the eggs were fertilized and regularly showed over 90 per cent normal cleavage. The rates on fertilized controls were also increased five times, which is difficult to reconcile with an initially high respiration of the unfertilized eggs due to injury. In the present work we have used even less shaking

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than recommended by Whitaker (50 per minute, 5.5 cm. arc), except for deliberate tests at higher rates. The protocol of such an experiment is given in Table I, and shows a minimal effect (not progressive with duration) with the fastest speed our shaker attains. Also, after several hours of fast shaking the same high fertilization (95 per cent) was obtained as at the start.

The measurement of egg quantity used is not a simple matter. Whitaker centrifuged his suspensions to constant egg volume in vaccine tubes (15 minutes, force not given) and, though realizing the possibility of errors due to imperfect packing, assumed these were small. He mentions no attempt to check this point. We counted the eggs in our suspensions with a hemocytometer and measured diameters with an ocular micrometer, from which data egg volumes per cubic centimeter of suspension are easily obtained. (In recalculating our data, Whitaker overlooked the given diameters and assumed a constant one of 74 micra which resulted in considerable discrepancies.) We had previously found centrifugation unsatisfactory in rough tests; now we have carefully compared both methods.

The measurement of egg diameters presents little difficulty. In good batches the majority of cells appear round and vary less than 10 per cent in individual measurements. Two observers, measuring different egg samples from the same suspension, regularly checked within 1.5 per cent. A typical result on fifteen eggs was: R.W.G.: 74.2 micra $\pm$ 0.3; B.B.R.: 73.8 $\pm$ 0.4. To the extent that eggs are slightly oblate and tend to settle on the flatter side, the egg volume calculated from the measured diameter will be high, and the rate of respiration calculated per unit volume correspondingly too low. This factor could hardly amount to 10 per cent and, if present, would make even our values below the correct ones.

Enumeration of the eggs is more difficult. Ideally a large counting chamber, holding up to a cubic centimeter of suspension, should give the best results. Actually, we have not found dilution followed by counting eggs in 0.5 cc. on a mechanical stage to be satisfactory. The ordinary blood-counting chamber, with 0.1 mm. between slide and cover-slip, permits counting the eggs in 0.9 c.mm. of undiluted suspension. The danger is that the large eggs settle so rapidly that all contained in a drop much thicker than 0.1 mm. may reach the slide surface before the cover-slip is placed and the fluid thinned. This does occur if the preparation is not made rapidly, and abnormally high (up to 30–50 per cent) counts result. The egg distribution per square is then likely to be very irregular. The further danger also exists that such a small sample is not fairly representative of the suspension. We have adopted a procedure of rapidly placing a drop, at

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once sliding on the cover-slip, and counting the four large corner squares (often the four side ones as well). Each observer counted five successive drops, the suspension being adequately mixed each time, and an average value taken. With practise, quite consistent results are obtained. The raw data of one run are given as an example (Table II).

The method has been further checked by counting a known suspension, diluting two and five times, and recounting. Results of such a test were: original suspension = 300,000/1 cc.; diluted two times = 295,000/2 cc.; diluted five times = 320,000/5 cc.

In six experiments, after obtaining the size and number of eggs in the suspension and calculating the volume of eggs per cubic centimeter, volume was directly measured on the centrifuge. Graduated centrifuge tubes were used and, though less accurate than vaccine tubes, agreed well in duplicate and certainly gave

TABLE II

Observer	Drop	Cells per 1 × 1 × 0.1 mm. corner square				Average	Mean and *P.E.*
B.B.R.	1	28	29	32	31	30.0	
	2	30	26	36	33	31.1	
	3	27	31	21	31	27.5	
	4	30	31	32	30	30.8	
	5	26	27	33	30	29.0	29.7 ± 0.5
R.W.G.	1	36	37	35	30	34.5	
	2	35	34	34	26	32.2	
	3	31	31	34	30	31.5	
	4	25	34	27	30	29.0	
	5	29	27	35	30	30.1	31.5 ± 0.6

approximately correct values. 2 to 5 cc. of suspension were used and spun for 1 to 10 minutes by hand or electric centrifuge. Although absolute constancy of volume was rarely attained, an approximate constancy was reached in 3 to 5 minutes. A volume that had become constant on hand centrifuging (radius 16 cm., 25 R.P.S.; centrifugal force = 400 × gravity) would shrink somewhat in the electric instrument under greater force (radius 18 cm., 32 R.P.S., C = 750 g.).

The ratio of minimum centrifuged volume to calculated one varied between 1.7 and 2.0. (In one case not included a ratio of 3.0 was obtained, but centrifugation was probably incomplete.) The average ratio was 1.8. That is: assuming the eggs were correctly counted and measured, the volume as determined by centrifuging was 80 per cent too high, and respiration rates calculated on such a basis correspondingly low.

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As an extra check on the centrifuge technique we used the Harvey centrifuge-microscope, kindly made available to us by Dr. E. N. Harvey. Eggs prepared as usual and the same after straining through two layers of 60 micra bolting-cloth to remove the jelly were used. At the slowest speed obtainable (higher than that usually used, C = 550 g.) the unfiltered eggs settled into a very loose mass. Individual eggs were rarely in contact with neighbors and often an egg radius separated them. This was largely due to the jelly, since the filtered eggs did come in contact and became deformed. Even here, however, many and large spaces were clearly visible at the edges of faceted surfaces. With still higher speeds (C = 1050 g., individual eggs were well stratified) the same pictures remained, though some further packing occurred. The conclusion seems clear that respirations calculated per unit volume, determined by the centrifuge, are subject to a large and variable error.

It will require further tests to determine the volume error for other eggs and cells. It is possible though unlikely (because of varying jelly masses, sizes, and toughness of membranes) that a uniform correction will apply to all of Whitaker's data, in which case the relation he has pointed out, that various eggs tend to the same respiratory rate after fertilization, will remain valid. Pending such controls, this must be regarded as uncertain; and the absolute values must surely be revised. It may be mentioned that data of other workers on different species of urchin eggs, which agree with Whitaker's values for *Arbacia*, were likewise based upon egg volumes determined on the centrifuge.

It remains to report the actual respiration rates obtained in the present experiments, carried out almost entirely according to Whitaker's procedure except for the measurement of egg volume. The Q_{O_2} per million eggs, in eleven experiments with three to six runs each, varied from 11.3 to 33.3. This is the same variability from batch to batch previously encountered. The average value at 21°C. was 19.5. In terms of the unit Whitaker has used, cubic millimeters O_2 per hour per 10 c.mm. of eggs, this becomes, for these eggs, 0.9. (Second decimals are not significant.) Whitaker obtained, also at 21°C., 0.4–0.5 for the unfertilized eggs. If this be corrected for the volume error of centrifuging (times 1.8) it becomes 0.7–0.9, in essential

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agreement with the above. Tang's result for unfertilized eggs at 24.7°C., in these units, is 1.4; Tang and Gerard's for fertilized eggs at 25°C., 6.1 (1/5 = 1.2 for the unfertilized equivalent). Applying a temperature coefficient of 4.0 to the unfertilized eggs, 2.0 to the fertilized (3), these values become, at 21°C., respectively 0.8 and (1/5 the fertilized value) 1.0. It is clear that all experimental series are in fair agreement when correct egg volumes are obtained and proper allowance made for temperature differences.

SUMMARY

Methods used in preparing *Arbacia* eggs for respiration studies, in carrying through the manometric determinations, and in estimating egg quantities have been reexamined.

Discrepancies in previous results are almost entirely due to a steady error in measuring egg volume by centrifuging. Volumes so obtained averaged 80 per cent too high.

The respiration of unfertilized eggs of *Arbacia punctulata* at 21°C. is 0.9 c.mm. O_2 per hour per 10 c.mm. of eggs.

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