ON THE ENERGETICS OF DIFFERENTIATION. VII

Comparison of the Respiratory Rates of Parthenogenetic and Fertilized Urechis Eggs

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The results of these experiments show that the rise in respiratory rate that occurs during development is correlated with cleavage; eggs that fail to cleave after activation show a greatly retarded rise while those that cleave show a rise that is roughly commensurate with their division rate. Inhibiting cleavage with phenylurethane affects the respiratory rate similarly.

Theoretical Part

It has been long been known that the rate of respiration rises during development. This increase in rate is evidently not directly proportional to the increase in the number of cells (*cf.* Needham, 1931). It might, nevertheless, depend upon changes in the egg brought about by cell division, so that when cleavage fails to occur the rise in respiration would be inhibited. We have considered in previous work the dependence of the form-changes on the respiration, the rate of oxygen consumption being taken as a measure of the energy available for the various developmental processes. We consider now the possibility that the developmental changes determine in turn the rate of respiration. If, for example, early cleavage is inhibited in a manner that does not affect the absolute rate of respiration at the particular stage, then we may expect, on this basis, failure of the subsequent rise.

In the early work of Warburg (1910) it has been shown that cleavage could be suppressed in sea urchin eggs by means of phenylurethane without immediately affecting the respiratory rate. However, the question of whether or not the rate would rise later was not investigated. Also it has been shown that after parthenogenetic activation of sea urchin eggs the same increase in rate occurs that is obtained normally upon fertilization (Warburg, 1910; Loeb and Wasteneys, 1913). But here again it would be desirable to know what happens later, especially since the parthenogenetically activated eggs develop much more slowly in general and often stop in early cleavage or even fail to divide.

In *Urechis* artificial activation with a single agent may produce dividing or non-dividing eggs depending on the length of treatment. Diluted sea water (Tyler, 1931) or ammoniacal sea water (Hiraiwa and Kawamura, 1936; Tyler and Bauer, 1937) may be used for this purpose.

Experimental Part

The respiration measurements were made by means of the usual Warburg method using the cylindrical type of vessel previously described (Tyler, 1936) but of 18 to 20 cc. calibration volume. The quantity of eggs present in each vessel was determined at the end of the run from the Kjeldahl nitrogen, and the oxygen consumption is expressed on that basis. The quantity of eggs employed was such as to give readings of 25 to 50 mm. Brodie fluid per hour at the start of the experiment.

The non-dividing and the dividing parthenogenetic eggs were produced simply by treatment with ammoniacal sea water, as previously described. Treatments of 2 to 7 minutes with 0.01 N NH_3 in sea water give 100 per cent activation with normal polar body extrusion but no cleavage. Such eggs go through a series of monaster cycles after polar body formation, but at a slower rate than would correspond to the normal nuclear changes. After about 10 to 15 hours unicellular swimmers may develop similar to the embryos differentiating without cleavage, first described by Lillie (1902). Treatments of 12 to 17 minutes with 0.01 N NH_3 in sea water give 100 per cent activation with as much as 90 to 100 per cent cleavage. Polar body formation is usually interfered with in such eggs, as previously described (Tyler and Bauer, 1937). After 8 to 12 hours swimming embryos including some normal ones may appear.

Non-Cleaving Parthenogenetic Eggs

The results of two sets of experiments with non-cleaving activated eggs are given in Table I, along with the fertilized controls. In both the treatment was for six minutes with 0.01 N NH_3, and insemination of the control was done at the start of the parthenogenetic treatment. Activation and fertilization were 100 per cent in both these experiments. As may be seen in Table I the oxygen consumption of the parthenogenetic eggs during the first hour of measurement is very nearly the same as that of the fertilized eggs. In both experiments the parthenogenetic eggs give slightly higher values, but the difference is no greater here than the difference between the duplicate vessels. During the subsequent hours the rate rises steadily in the case of the fertilized eggs. The parthenogenetic eggs, however, show first a

slight drop (second to fourth hours) followed by an increasing rate. After 8 hours the parthenogenetic eggs attain a rate that is about double their initial respiration, but only about half of that of the fertilized eggs.

The eggs from the vessels were examined at the end of the run. In both experiments the parthenogenetic eggs gave about 2 per cent of unicellular swimmers while the fertilized eggs gave 90 to 98 per cent normal top-swimming trochophores.

Cleaving Parthenogenetic Eggs

The respiration data of two sets of experiments with eggs that divide after ammonia treatment are given in Table II. In both

TABLE I

Oxygen consumption of non-cleaving parthenogenetic eggs. Parthenogenetic treatment = 6 minutes with 0.01 N NH_3 in sea water. Measurements begun at 43 minutes after treatment or insemination in Experiment I and 60 minutes after in Experiment II. Values given as cu. mm. O_2 per hr. per mg. N. Temperature = 22.0° C.

Experiment	I				II		
Hour	Parth.	Parth.	Fert.	Fert.	Parth.	Fert.	Fert.
1st	4.64	4.48	4.38	4.24	4.72	4.53	4.14
2nd	4.40	4.30	4.53	4.38	3.88	5.05	4.49
3rd	4.02	3.99	5.60	5.55	3.83	5.45	5.35
4th	4.52	4.34	7.58	7.53	4.32	7.01	6.65
5th	5.22	5.07	10.01	9.93	5.56	10.10	10.20
6th	6.19	6.02	13.60	13.43	7.40	13.83	13.48
7th	6.86	7.29	16.38	16.31	9.66	18.26	17.45
8th	8.91	8.87	18.66	18.47	10.93	21.64	20.81

experiments it may be seen that the respiration of the parthenogenetic eggs at the start is slightly higher than that of the fertilized controls. A third set not presented in the table shows the same initial difference, and also agrees very well throughout the run. The difference here is somewhat greater than was manifested in the experiments with the non-cleaving parthenogenetic eggs. It may be pointed out that the ammonia treatment is considerably longer here (13 to 17 minutes) and this may account for the higher initial rate.

The subsequent readings give values that are fairly constant for the parthenogenetic eggs up to the fifth hour, while the fertilized eggs rise as usual during this time. Following this the rate rises and at the eighth hour the respiration is about half of the control rate. It is very nearly the same at that time as is obtained with the non-cleaving

parthenogenetic eggs (Table I). However, differences appear later as will be shown below.

The cleavage of these parthenogenetic eggs is very slow compared with the fertilized controls. In Experiment I, at 2 hours after treatment, there were 43 per cent cleaved of which 15 per cent were in two cells, 22 in three and 6 in four. The fertilized controls were 100 per cent in four at this time. An hour later there was 75 per cent cleavage. At the end of the run (11 hours after treatment) the eggs from the vessels were 90 per cent cleaved and 30 per cent were swimmers.

In Experiment II, 60 per cent of the parthenogenetic eggs were divided at two hours after treatment, the distribution being 23 per cent

TABLE II

Oxygen consumption of cleaving parthenogenetic eggs. Parthenogenetic treatment = 13 minutes (Experiment I) and 17 minutes (Experiment II) with 0.01 N NH_3 in sea water. Measurements began at 74 minutes after treatment or insemination in Experiment I and 53 minutes after in Experiment II. Values given as cu. mm. O_2 per hour per mg. N. Temperature = 22° C.

Experiment	I				II			
Hour	Parth.	Parth.	Fert.	Fert.	Parth.	Parth.	Fert.	Unfert.
1st	5.88	5.81	4.48	4.64	5.69	5.70	4.51	3.69
2nd	5.45	5.69	4.92	4.82	5.40	5.48	4.60	3.90
3rd	5.20	5.25	5.63	5.80	5.51	5.34	5.59	3.37
4th	5.36	5.36	7.03	6.96	5.85	5.70	6.94	——
5th	5.63	5.25	10.28	10.12	5.91	5.48	9.07	3.29
6th	5.99	5.92	14.05	13.80	7.01	6.86	13.17	3.37
7th	7.55	7.25	17.46	17.83	8.04	8.02	16.27	
8th	8.62	8.04	20.90	21.14	10.25	10.18	20.06	
9th	9.71	9.49	24.50	24.45				
10th	10.65	10.95	——	28.55				

in two cells, 30 in three and 7 in four. The fertilized controls were all in four cells at the time. At the end of the run (10 hours) the parthenogenetic eggs from the vessels were 95 per cent cleaved of which 5 per cent were swimmers, while the fertilized eggs gave 90 per cent swimmers.

Since the parthenogenetic eggs do not divide at all synchronously and since many may stop after one or more cleavages, the distribution becomes quite complicated. It is clear, however, that cleavage and the development of cilia are retarded.

Later Stages

Measurements on the later stages were made by culturing the eggs in dishes and washing the embryos before transfer to the vessels.

This is to avoid possible effects of bacterial growth or other changes produced in the vessels during a prolonged run. In Table III the results of one set of experiments are given. Cleaving and non-cleaving parthenogenetic eggs were prepared from the same batch along with the fertilized controls, and allowed to develop for 11 hours at room temperature (18.2° C.) before transfer to the vessels. In the fertilized lot there were more than 95 per cent top-swimming young trochophores, and only the top swimmers were transferred to the vessels. In the non-cleaving parthenogenetic lot (Parth. 5) there were no swimmers at that time. In the cleaving parthenogenetic lot (Parth. 17) 95 per cent had divided and practically all of them were bottom-swimmers.

TABLE III

Oxygen consumption of late stages of non-cleaving and cleaving parthenogenetic eggs. Parthenogenetic treatment = 5 minutes (Parth. 5) and 17 minutes (Parth. 17) with 0.01 N NH_3 in sea water. Eggs cultured for 11 hours at 18.2° before measurements were begun. Values given as cu. mm. O_2 per hour per mg. N. Temperature = 22°.

Hour	Fert.	Fert.	Parth. 5	Parth. 5	Parth. 17	Parth. 17
1st	21.80	22.09	12.31	12.17	11.64	11.69
2nd	25.45	25.91	12.54	12.42	19.03	18.45
3rd	29.38	30.48	15.11	14.90	18.74	18.96
4th	32.39	33.32	15.01	14.65	20.75	20.26
5th	36.17	36.18	14.44	14.15	22.60	22.35
6th	39.94	40.48	15.76	15.72	24.76	24.98
7th	43.31	45.70	18.98	18.75	27.70	27.95
8th	46.50	47.70	22.18	22.73	31.73	32.67

As may be seen from Table III the two types of parthenogenetic eggs respire at very nearly the same rate at the start of the experiment. The rate is a little more than half of the fertilized rate at this time. The rate rises with time, but more rapidly in the case of the dividing eggs. At the end of the eight-hour period they are respiring at almost one and a half times the rate of the non-cleaving eggs, but still only two-thirds that of the fertilized controls. Examination of the eggs from the vessels at the end of the run showed 20 to 30 per cent bottom-swimmers (unicellular) in the case of the non-cleaving eggs (Parth. 5); 100 per cent swimmers of which 30 to 40 per cent were top-swimmers in the case of the cleaving eggs (Parth. 17); and 100 per cent top-swimmers in the case of the fertilized controls.

Fertilized Eggs in Phenylurethane

With the proper concentration of phenylurethane, cleavage may be suppressed without the initial respiratory rate being affected, as

Warburg (1910) showed on sea urchin eggs. The experiments were repeated for the purpose of covering a longer period. The results of such an experiment with *Urechis* eggs are presented in Table IV. The eggs were placed in the solution at 45 minutes after insemination, and the measurements begun 40 minutes later. As the figures in Table IV show, the initial rate of respiration is the same as in the untreated controls. The rate then rises in both, but more slowly in the treated eggs. At the end of the run their respiration is less than three-fourths of the control rate.

It is evident here that the failure of cytoplasmic cleavage is accompanied by a slower rise in respiratory rate. The rate does, however, actually rise. It may be pointed out in this connection that nuclear division goes on in the treated eggs but at a retarded rate.

TABLE IV

Oxygen consumption of phenylurethane-treated eggs. Eggs placed in 5×10^{-4} N phenylurethane after appearance of second polar body (45 minutes after insemination). Measurements begun at 85 minutes after insemination and 40 minutes after immersion in phenylurethane. Values given as cu. mm. O_2 per hour per mg. N. Temperature = 22.0° C.

Hour	Control	Control	Phenylur.	Phenylur.
1st	4.61	4.64	4.86	5.04
2nd	4.94	4.92	4.69	4.83
3rd	5.66	5.48	5.40	6.71
4th	6.99	7.05	6.72	5.13
5th	9.84	10.02	9.16	10.84
6th	13.34	13.54	10.80	9.26
7th	16.30	16.40	12.30	14.01
8th	18.83	18.79	13.68	14.01
9th	21.61	21.33	15.43	15.98

Runnström (1928) investigated the action of phenyl- and ethylurethane on the respiration of sea urchin eggs. The concentrations that he used gave a depression of the initial rate; nevertheless a distinct rise is manifested during the three-hour period of the experiments. He notes too that nuclear division proceeds although the respiration during the first hour may be only 35 per cent of that of the control.

DISCUSSION

The curves of Fig. 1 summarize the results. It is readily seen that the parthenogenetic eggs, although starting out at about the same rate as the fertilized eggs, do not give as rapid a rise with time. The two types of parthenogenetic eggs give approximately the same values during the early stages, but later the dividing eggs manifest a

more rapid rise. If only the early period were considered this might be taken to mean that the rise in respiratory rate is not at all correlated with cleavage. However, as pointed out above, the cleavage parthenogenetic eggs divide at a retarded rate. Also, it is evident that in normal fertilized eggs no appreciable rise occurs during the early cleavage. It is therefore fairly safe to conclude that the rise in rate is linked with cleavage. But that it is not merely a matter of cytoplasmic cleavage is clear from the fact that in the non-cleaving parthenogenetic eggs and the phenylurethane-treated eggs the respiratory rate does rise with time. Here, as we have seen, nuclear division goes on and it is probably with this factor that the rise is connected.

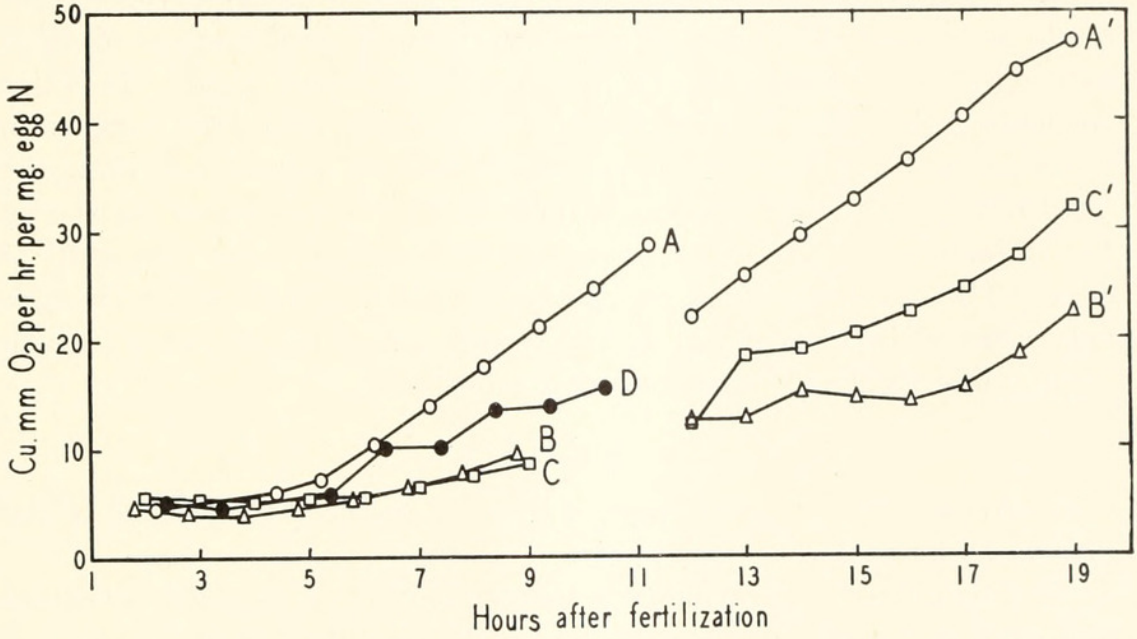

FIG. 1. Rate of oxygen consumption of *Urechis* eggs. Curves *A* and *A′*; fertilized eggs. *B* and *B′*; non-cleaving parthenogenetic eggs. *C* and *C′*; cleaving parthenogenetic eggs. *D*; phenylurethane-treated eggs. Temperature 22° C. Values are averages from all of the data. *A′*, *B′* and *C′* are not direct continuations of *A*, *B*, and *C* since the eggs had been cultured at 18.2° C. for 11 hours.

Similar experiments have recently been independently performed by Brachet (1938) on *Chætopterus* eggs that "differentiate without cleavage" and he has kindly allowed us to examine his manuscript. The results with *Chætopterus* agree very well with those presented here. In addition Brachet has investigated the nucleic acid content and finds it to be much lower in the parthenogenetic (non-cleaving) eggs than in the fertilized controls.

In experiments of this type it is, of course, important to rule out possible direct effects of the chemical agent employed. It is reasonable to assume that there has been no direct effect when the initial rate is unaltered. Such is the case in the experiments reported here with

the exception of the cleaving parthenogenetic eggs (Table II) which showed a significantly high initial respiratory rate. This is very likely due to the ammonia treatment and from the subsequent values we might assume that the effect passes off. In any event, the difference would have to be greater and in the opposite direction to seriously affect the conclusion.

As was pointed out in the introduction, we might expect no rise in respiration if cleavage were blocked by some means that does not alter the absolute respiratory rate at the particular stage. But we are not dealing here with such ideal cases. In these experiments cytoplasmic division fails (non-cleaving parthenogenetic eggs) or is prevented (phenylurethane-treated eggs) while nuclear division proceeds at a retarded rate; or both cytoplasmic and nuclear division proceed at a retarded rate (cleaving parthenogenetic eggs). It is not surprising, therefore, to find that the respiratory rate does increase in these cases. That the parthenogenetic show a slower rise than the fertilized eggs is consistent with their slower development, as is also the difference between the phenylurethane-treated and the control eggs. The difference between the two types of parthenogenetic eggs may likewise be interpreted as due to differences in the rate at which comparable stages of development are reached.

Summary

1. Artificially activated eggs of *Urechis* respire at the same initial rate as do normally fertilized eggs.

2. The rate of respiration rises with time in the artificially activated eggs, but at a much slower rate than in the fertilized eggs.

3. The increase in respiratory rate with time is greater with cleaving than with non-cleaving parthenogenetic eggs.

4. Fertilized eggs in which cytoplasmic cleavage is inhibited and nuclear division retarded by means of phenylurethane give a retarded rise in respiratory rate, although the initial rate is the same as the control rate.

5. It is concluded that the delayed rise in respiration is linked with the slower development in all these cases.

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