ATP level and respiration of embryos

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A considerable increase in the rate of oxygen consumption is known to occur during the development of embryos (cf. Needham, 1931; Tuft, 1953; Brachet, 1960; Gustafson, 1965). However, the mechanism of the increase in respiration during embryonic development is still unclear. A certain correlation seems to exist between DNA synthesis and the respiration of embryos (Comita & Whiteleley, 1953; Brachet, 1960). According to Commoner (1964), this correlation is determined by a change in the level of free nucleotides in cells when the rate of DNA synthesis changes. Free nucleotides control cell metabolism, oxidative processes in particular. The ADP+P/ATP ratio is known to control the rate and direction of electron transfer in the respiratory chain (Chance & Hagihara, 1961; Klingenberg & Schollmeyer, 1961). The ADP/ATP system is suggested to control the rate of oxidative metabolism in fertilization (Monroy, 1965a, b; Zotin, Milman & Faustov, 1967).

The work described below was aimed at the elucidation of the role played by ATP in respiration changes of embryos during their development and during changes in environmental temperature.

MATERIALS AND METHODS

Experiments were carried out on sea-urchin eggs (Strongylocentrotus drobachiensis O. F. Müller) in the Murmansk Marine Biological Institute, and on embryos of loach (Misgurnus fossilis L.), axolotl (Ambystoma mexicanum L.), frog (Rana temporaria L.) and toad (Bufo viridis L.) in Moscow. Loach eggs were obtained by choriogonin injection of females, and reared at 15–16 °C. Axolotl, frog, toad and sea-urchin eggs were taken from naturally matured females and reared at 5–8 °C (sea urchin) and 16–18 °C (axolotl, frog, toad). Samples for the measurements of the ATP concentration in the eggs of loach, axolotl, frog and toad were prepared in the following matter: 20 loach eggs in membranes or 10 amphibian eggs devoid of the jelly envelope were homogenized in the cold in 3 ml of double-glass distilled water; the homogenizer was rinsed with

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3 ml of water, the homogenate plus rinse heated in a boiling-water bath for 7 min, cooled and stored frozen. Sea-urchin eggs were fixed by the method described earlier (Zotin, Milman & Faustov, 1965). The ATP level was determined in the samples by the method of McElroy & Strehler (1957). A crude extract of the lanterns of *Luciola mingrelica* in 0·07 M tris was used as a luciferin-luciferase system. Luminescence was measured on a specially constructed apparatus (Milman & Danyukov, 1965).

In order to check whether ATPase caused ATP hydrolysis during homogenization (for 1 min) we have carried out preliminary experiments on loach and axolotl embryos. As can be seen from Table 1, storage of the embryo homogenates in the cold did not lead to significant changes of their ATP content.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage of Development</th>
<th>1·0 min</th>
<th>1·5 min</th>
<th>2·0 min</th>
<th>2·5 min</th>
<th>3·0 min</th>
<th>4·0 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axolotl</td>
<td>Early blastula</td>
<td>5·2</td>
<td>5·2</td>
<td>5·2</td>
<td>4·6</td>
<td>4·9</td>
<td>4·6</td>
</tr>
<tr>
<td>Axolotl</td>
<td>Hatching</td>
<td>3·6</td>
<td>3·9</td>
<td>3·6</td>
<td>3·4</td>
<td>3·6</td>
<td>3·6</td>
</tr>
<tr>
<td>Loach</td>
<td>Four blastomeres</td>
<td>0·58</td>
<td>—</td>
<td>0·52</td>
<td>—</td>
<td>0·58</td>
<td>0·58</td>
</tr>
</tbody>
</table>

The effect of temperature upon respiration and ATP level was studied in loach eggs. An hour after insemination 300–400 eggs were placed into each of several Warburg flasks. After an hour, oxygen consumption was measured during 3 h. The temperature in the bath of the Warburg apparatus was maintained constant to within 0·1 °C during the experiment. To study the effect of temperature upon ATP level, an hour after insemination loach eggs were placed in homogenizers (40 eggs per batch) in 0·5 ml of water, and the homogenizers were immersed in water baths and the temperature was maintained constant by Wobser ultrathermostats within 0·1 °C. After 2 h in the water bath the eggs were rapidly frozen in liquid nitrogen, thawed and fixed. Then their ATP content was determined as mentioned above.

RE SULTS

Changes in the ATP level after fertilization

The suggestion that high energy phosphate compounds of the ATP type play an important role in the fertilization process has been made many times. However, Chambers & Mende (1953) did not find significant differences in adenylnucleotide content of sea-urchin and starfish eggs before and after fertilization. Data recently published report that the ATP level falls while that of ADP increases after fertilization of sea urchin eggs (Monroy, 1965a, b).
ATP level and respiration

The ATP level in the eggs of *Strongylocentrotus drobachiensis* was determined 10, 40 and 60 min after fertilization. As can be seen from Table 2, the drop in the ATP level in fertilized eggs compared to unfertilized ones, occurs mainly during the first minutes after fertilization. Taking into consideration all the data obtained, the ATP level decreases after fertilization by $16.6 \pm 2.7\%$ on average.

It was shown previously that the ATP level in fertilized *S. drobachiensis* eggs is on average $1.50 \mu g$ ATP/1000 eggs (Zotin et al. 1965). Therefore, after fertilization the ATP level drops by $0.30 \mu g/1000$ eggs.

Table 2. *Decrease in the ATP level in sea-urchin eggs after fertilization*

<table>
<thead>
<tr>
<th>Time after fertilization (in min)</th>
<th>ATP level decrease</th>
<th>No. of measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/1000 eggs</td>
<td>%</td>
</tr>
<tr>
<td>10</td>
<td>0.25</td>
<td>13.9</td>
</tr>
<tr>
<td>40</td>
<td>0.31</td>
<td>17.3</td>
</tr>
<tr>
<td>60</td>
<td>0.37</td>
<td>21.0</td>
</tr>
</tbody>
</table>

Table 3. *Changes in the ATP level in seven egg clutches of loach after activation in water*

<table>
<thead>
<tr>
<th>ATP level (µg/egg)</th>
<th>Non-activated eggs</th>
<th>Activated eggs</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.60</td>
<td>0.58</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>0.55</td>
<td>0.52</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>0.57</td>
<td>0.52</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>0.48</td>
<td>0.56</td>
<td>-16.6</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.52</td>
<td>-4.0</td>
</tr>
<tr>
<td></td>
<td>0.54</td>
<td>0.46</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>0.52</td>
<td>0.51</td>
<td>2.0</td>
</tr>
</tbody>
</table>

We failed to find similar changes in the ATP level in fertilized or activated and non-activated loach eggs. In order to compare the ATP level in fertilized and unfertilized loach eggs, unfertilized eggs had to be washed from the perivisceral fluid. Unfertilized eggs of teleost fishes are known to be activated in water. To prevent activation they were washed in Holtfreter solution of treble concentration. Fertilized or activated eggs were also washed with this solution before fixation. Experiments were performed mainly on water-activated loach eggs. In two experiments the ATP level was determined in fertilized eggs 30 min after insemination. It did not differ from that in activated eggs. Table 3 presents the results of the determination of the ATP level in seven clutches of activated and non-activated eggs. As can be seen from these determinations, there is no difference in the ATP level between activated and non-activated loach eggs.
Changes in the ATP level during the development of embryos

The ATP level was determined on three egg clutches taken from different sea urchins. The mean data of these determinations are presented in Fig. 1. After fertilization, at the first cleavage stages the ATP level remained unchanged. After the 64-cell stage, the ATP level rapidly dropped until hatching (the mid-blastula stage). During subsequent development the ATP level changed but little, remaining at 35–40% of that at the first cleavage stages. As mentioned earlier, *S. drobachiensis* eggs contain about 1–50 μg ATP/1000 eggs at early cleavage stages. Therefore, by the hatching stage when the ATP level reached its minimum, it drops to 0.52–0.60 μg/1000 eggs.

![Graph showing changes in ATP level and respiration rate during development](image)

Fig. 1. Changes in the ATP level (1) and respiration rate (2) during development of sea-urchin embryos. The data on respiration are taken from the paper of Tyler & Humason, 1937. b, 64-cell blastomere stage; h., hatching; o.g., onset of gastrulation.

It would be of interest to compare the changes in the ATP level in sea-urchin embryos with the changes in the respiration rate during development. No data could be found in the literature concerning respiration of *S. drobachiensis* embryos, so that the data on the respiration of *S. purpuratus* obtained by Tyler & Humason (1937) were used. The ATP level and respiration were compared by developmental stages. As may be seen from Fig. 1, the rate of oxygen consumption by sea-urchin eggs increases as the ATP level falls.

Experiments were carried out on five axolotl egg clutches. In all instances similar results were obtained, as summarized in Fig. 2. Determinations of the
ATP level and respiration

ATP level in axolotl eggs were started on the second day after oviposition, when the embryos were at the blastula stage (stage 8 of Harrison). A measurement of the absolute ATP content showed that at that time the embryos contained 5.80 \( \mu g \) ATP per egg. The ATP level continuously dropped during the development, and at hatching was 65–70 % of that at mid-blastula, i.e. 3.77–4.06 \( \mu g \) ATP/embryo. To compare the respiration rate with the changes in the ATP level in axolotl embryos data on the respiration of embryos at various developmental stages are presented in Fig. 2 (after Løvtrup, 1953). As may be seen from this comparison, a decrease in the ATP content in axolotl embryos during development is accompanied by an increase in the rate of oxygen consumption.

Fig. 2. Changes in the ATP level (1) and respiration rate (2) during the development of axolotl embryos. The data on respiration are taken from the paper of Løvtrup (1953).

Only preliminary data were obtained on the change in the ATP level and respiration in frog and toad embryos, since only one egg clutch of each of the species was available. The ATP level in toad embryos dropped by 20 % from the 4-cell blastomere stage to hatching, or, in absolute terms, from 1.02 to 0.82 \( \mu g \)/embryo, i.e. by 0.20 \( \mu g \) ATP/embryo. Simultaneously with the drop in the ATP level an increase in the rate of oxygen consumption occurred in the toad embryos (Fig. 3). In frog embryos from the 4-cell blastomere stage to hatching the ATP level decreased by 26 % or, in absolute terms, from 1.90 \( \mu g \)/embryo to 1.40 \( \mu g \)/embryo, i.e. by 0.50 \( \mu g \)/embryo. Simultaneously with the drop in ATP in *R. temporaria* embryos, the rate of oxygen consumption increased (cf. Ten Cate, 1956).

In loach embryos the ATP level was determined in eight egg clutches. The average date of these experiments are depicted in Fig. 4. After fertilization the ATP level continuously dropped in the eggs until hatching, decreasing by
Fig. 3. Changes in the ATP level (1) and respiration rate (2) in toad embryos.

Fig. 4. Changes in the ATP level (1) and respiration rate (2) during the development of loach embryos. Data on respiration are taken from the paper of Neyfakh (1960).
ATP level and respiration

30 % of the initial level. To determine the statistical significance of the decrease in ATP level, in one clutch of loach eggs the ATP level was determined in twenty parallel series at the stage of the second division and at hatching. The ATP level dropped from 0·62 ± 0·037 μg/embryo at the stage of cleavage to 0·42 ± 0·013 μg/embryo at hatching, i.e. by 31·5 %. Milman & Danyukov (1965) showed one loach egg to contain about 0·50 μg ATP at the cleavage stage. According to our determinations performed on the eggs taken from 12 loach females, the ATP level is on average 0·57 μg/egg at the cleavage stage. On the basis of these determinations and assuming that during the development from fertilization to hatching the ATP level in loach embryos decreases by 30 %, it can be calculated that at hatching the ATP level decreases by 0·17 μg/embryo. As can be seen from Fig. 4, the drop in the ATP level during the development of the loach embryo is accompanied by an increase in the oxygen consumption rate. The data on respiration of the loach embryo were taken from the paper of Neyfakh (1960).

Table 4. ATP level in different cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>ATP level (% of dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleaving eggs:</td>
<td></td>
</tr>
<tr>
<td>Sea urchin</td>
<td>0·27</td>
</tr>
<tr>
<td>Axolotl</td>
<td>0·27</td>
</tr>
<tr>
<td>Frog</td>
<td>0·16</td>
</tr>
<tr>
<td>Toad</td>
<td>0·24</td>
</tr>
<tr>
<td>Loach</td>
<td>0·18</td>
</tr>
<tr>
<td>Ehrlich ascite carcinoma</td>
<td>0·16</td>
</tr>
<tr>
<td>Fibroblast culture</td>
<td>0·12</td>
</tr>
<tr>
<td>Adult loach:</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0·034</td>
</tr>
<tr>
<td>Liver</td>
<td>0·046</td>
</tr>
<tr>
<td>Muscle</td>
<td>0·013</td>
</tr>
<tr>
<td>Adult mouse:</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0·0017</td>
</tr>
<tr>
<td>Liver</td>
<td>0·0012</td>
</tr>
<tr>
<td>Kidney</td>
<td>0·0015</td>
</tr>
<tr>
<td>Spleen</td>
<td>0·0016</td>
</tr>
</tbody>
</table>

ATP level and frequency of cell division

As may be seen from Figs. 1 to 4, in echinoderm, fish and amphibian eggs the ATP level gradually decreases with development. When calculated per dry weight from the first divisions to hatching, the ATP level in sea-urchin embryos decreases by from 0·27 to 0·10 %, in axolotl embryos by from 0·27 to 0·15%, in loach embryos by from 0·18 to 0·12 %, in frog embryos by from 0·16 to 0·11 % and in toad embryos by from 0·24 to 0·18 %. At subsequent developmental stages this decrease might be still greater. For example, in axolotl larvae 20 days after hatching the ATP level is 0·11 % of dry weight of the embryos. These data suggest that
the high ATP level is characteristic of the period of rapid cell division in embryonic
development. In order to investigate this further the ATP level was determined
in Ehrlich ascite carcinoma cells, Chinese hamster fibroblasts (cell culture),
and in liver, muscle and brain of the adult loach and albino mouse (Table 4).
As may be seen in brain, liver and kidney where the percentage of dividing
cells is not high, the ATP level is considerably lower than in rapidly dividing
cells of cleaving eggs, Ehrlich carcinoma and fibroblast culture.

Temperature effect upon the ATP level and the
respiration of embryos

Temperature effect upon the respiration and the ATP level in loach embryos
was studied during the first cleavage divisions, as it is known that during the
first five to six divisions of loach eggs the oxygen consumption rate remains
unchanged (Neyfakh, 1960). The same is true for the ATP level in loach eggs
during cleavage (Milman & Danyukov, 1965).

In all, about 140 determinations of the ATP level were carried out on the eggs
taken from 27 loach females, and respiration rate (180 determinations) was
measured on eggs taken from 50 females. The data obtained are shown in Fig. 5.
The curves were obtained by treating the data by the 'sum of least squares'
method. The formula \( Q = a(t^o - c)^b \) was used for respiration, when \( Q \) is the
amount of oxygen consumed in mm\(^3\)/h/embryo, \( t^o \) is the temperature in °C, and
\( a, b \) and \( c \) are constants. The ATP concentration follows a straight-line equation.
Starting from a temperature of 25 °C the percentage of embryo mortality markedly
increases, attaining about 40 % at 30 °C. Dead eggs do not respire so that a
Correspondingly correction was introduced when calculating oxygen consumption, especially at high temperatures.

As can be seen from Fig. 5, the respiration rate of cleaving loach eggs increases with changing of temperature from 0.0055 mm³/h/embryo at 4 °C to 0.0909 mm³/h/embryo at 30 °C. The ATP level drops from 0.64 µg/egg at 4 °C down to 0.40 µg/egg at 30 °C. Thus for the increase in the rate of oxygen consumption of loach embryos with rising temperature, as well as in the case of the change in respiration in embryos during development, the increase is accompanied by a drop in ATP level.

**DISCUSSION**

Various hypotheses have been suggested to explain the mechanism of control of respiration during embryonic development. An increase in respiration during the development of sea-urchin embryos may be controlled at the first stage (during cleavage) by the enzymic activity of the hexose monophosphate shunt, and by an increase in the number of mitochondria at the second stage (gastrulation) (Gustafson, 1965). This suggestion, however, cannot be applied to all other animal species, since the number of mitochondria does not increase during the development of some species of amphibians (Brachet, 1960) and teleosts (Abramova, Likhtman & Neyfakh, 1965). It has also been shown that the activity of cytochrome-oxidase (Brachet, 1960), NADH-cytochrome reductase (Radzinskaja, 1967) and of some other oxidative enzymes is not the limiting factor of respiration in amphibian and fish embryos. Neither do respiration substrates limit oxygen consumption (Abramova et al. 1965). The suggestion of Immers & Runnström (1960) and then other authors (Monroy, 1965a; Zotin et al. 1967) that the respiration level of embryos is controlled by the ADP/ATP ratio seems to be the most likely one. At any rate, observations described in this paper do not contradict this suggestion. In fact, after fertilization of sea-urchin eggs, a considerable increase in respiration is known to occur (cf. Rothschild, 1956; Brachet, 1960; Monroy, 1965a, b). Simultaneously, a drop of the ATP level (Table 2) and of the ATP/ADP ratio occurs (Monroy, 1965a). In teleost eggs no respiration increase is observed after fertilization (Nakano, 1953; Rothschild, 1956); a drop of the ATP level also does not occur (Table 3). Many authors have demonstrated that at the first stages of embryo cleavage no marked increase in respiration rate occurs (Lindahl, 1939; Løvtrup, 1953; Tuft, 1953; Neyfakh, 1960); the ATP concentrations in the eggs of sea urchins (Nilsson, 1961; Taguchi, 1962a; Epel, 1963; Zotin et al. 1965) and teleosts (Taguchi, 1962b; Milman & Danyukov, 1965) changes but little. On the contrary, as may be seen from the data presented in this paper, as well as from those obtained by other authors (Hultin, 1957; Nilsson, 1961; Taguchi, 1962a) during late stages of development, respiration increases while the ATP level in the embryos drops as development proceeds. Finally, an increase in
oxygen consumption by loach embryos with increasing temperature is also accompanied by a drop in the ATP level. These observations seem to support the suggestion of the role played by the ADP/ATP ratio in the respiration control of the embryos.

Data are available demonstrating the dependence of the respiration level on the rate of DNA synthesis in the embryos (Comita & Whiteleley, 1953; Brachet, 1960). According to the scheme presented by Commoner (1964) the following relation takes place:

\[
\text{DNA/cell} \rightarrow \text{amount of DNA synthesized/cell} \rightarrow \text{free nucleotide level} \rightarrow \text{rate of oxidative metabolism.}
\]

This scheme may serve as the basis for the elaboration of a theory of the regulation mechanism of embryo respiration. In our opinion, it is the changes in the ADP/ATP ratio in the embryos due to changes in DNA synthesis that underlie this mechanism, so that a blockage of DNA synthesis in sea-urchin eggs results in a sharp increase in ATP level (Zotin et al. 1965).

The discussion of the role of ATP in embryonic development poses some other problems as well. In particular, it has been shown that in the period of cleavage the ATP level is much higher than during successive developmental stages and than in tissues where the frequency of cell division is not high. It can be suggested therefore that the ATP level is associated with the regulation of the rate of cell division. This regulation occurs through a change in the ADP/ATP ratio in the cells which leads to a change in the rate of respiration and glycolysis, and to an activation of cell division.

**SUMMARY**

1. During the first stages of cleavage in sea-urchin embryos their ATP level remains constant. Starting from the 64-cell stage, the ATP level rapidly drops attaining the minimal value by hatching. During this period the ATP level decreases from 1.50 to 0.52–0.60 μg/1000 embryos.

2. During the development of axolotl, frog, toad and loach embryos from the stage of cleavage to that of hatching, a continuous drop in the ATP level occurs from 5.80 to 3.77–4.06 μg/embryo in axolotls, from 1.90 to 1.40 μg/embryo in frogs, from 1.02 to 0.82 μg/embryo in toads and from 0.57 to 0.40 μg/embryo in loaches.

3. A comparison of the ATP level in rapidly dividing cells (cleaving eggs, Ehrlich carcinoma, fibroblast culture) with the cells of loach muscle, liver and brain, and mouse kidney, liver and brain, shows that dividing cells are characterized by a raised ATP level.

4. The respiration rate of cleaving loach eggs during a temperature rise from 4 to 30 °C increases from 0.0055 to 0.0909 mm³/h/embryo, the ATP level in the embryo decreasing simultaneously from 0.64 to 0.40 μg/embryo.
АТФ уровень и дыхание

5. Сравнение изменений в уровне АТФ и дыхания во время развития зародышей и при изменении температуры позволяет высказать предположение, что изменение интенсивности дыхания зародышей во время развития контролируется соотношением АТФ/АДФ.

Выводы

1. Во время первых делений дробления яиц морского ежа уровень АТФ остается постоянным. Начиная со стадии 64 бластомер уровня АТФ в яйцеклетках быстро падает, достигая минимальной величины к стадии выкл congratulateся. За этот период содержание АТФ в яйцах падает с 1,50 до 0,52–0,60 мкг в 1000 яиц.

2. Во время развития аксолотля, лягушки, жабы и вьюна от стадии дробления до выкл congratulateся происходит непрерывное уменьшение содержания АТФ в зародышах: от 5,80 до 3,77–4,06 мкг/яйцо; от 1,90 до 1,40 у лягушки; от 1,02 до 0,82 у жабы и от 0,57 до 0,40 у вьюна.

3. Сопоставление содержания АТФ в интенсивно делящихся клетках (дробящиеся яйца, асцитный рак Эрлиха, культура фибробластов) с такими тканями, как мышцы, печень, мозг и почки вьюна и печень и мозг белой мыши показало, что активно делящиеся клетки характеризуются более высоким уровнем содержания АТФ (0,12–0,27% от сухого веса).

4. Интенсивность дыхания дробящегося яйца вьюна возрастает при увеличении температуры от 4 до 30 °C с 0,0055 до 0,0909 мм³/час/яйцо, а уровень АТФ при этом падает с 0,64 до 0,40 мкг/яйцо.

5. Сравнение изменения уровня АТФ и дыхания во время развития зародышей и при изменении температуры позволило высказать предположение, что изменение интенсивности дыхания зародышей во время развития контролируется соотношением АДФ/АТФ.

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