THE METABOLISM OF THE SEA-URCHIN EGG

ANAEROBIC BREAKDOWN OF CARBOHYDRATE

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(With One Text-figure)

INTRODUCTION
Although a knowledge of resting egg metabolism is essential for an understanding of
the changes in metabolism which occur at fertilization, opinions are still divided
regarding the pathways of carbohydrate breakdown in the sea-urchin egg. A detailed
examination of these differences of opinion has recently been published (Rothschild,
1951), so here it need only be said that the Swedish school, notably Lindberg &
Ernster (1948), believe that in the sea-urchin eggs, carbohydrate is mainly broken
down by an oxidative mechanism known as the hexose monophosphate shunt
(Warburg & Christian, 1932; Dickens, 1936, 1938a, b) and not through the classical
glycolytic pathways. The evidence in favour of this hypothesis cannot be considered
conclusive, while Ycas (1950) has described experiments which support the opposite
view, that sea-urchin egg carbohydrate is broken down by the conventional glycolytic
mechanisms. The object of the work described in this paper was to try and resolve
these differences of opinion, using the unfertilized eggs of Echinus esculentus. The
eggs of E. esculentus will be shown to contain active glycolytic enzymes and to
produce lactic acid under anaerobic conditions.

MATERIAL AND METHODS
Eggs were obtained from the ovaries by one of two methods: (a) Separated ovaries
were cut up and the contents suspended in about 5 vol. of sea water by gentle
agitation. The suspension was strained through bolting silk, and centrifuged; the
supernatant was removed by suction and the eggs were resuspended in about
50 vol. of sea water. After the eggs had settled, the supernatant was removed by
suction. (b) Normal and rapid extrusion of the eggs through the genital pores was
induced by placing cotton-wool soaked in 0.5 M-KCl on the ovaries in situ. These
eggs were centrifuged and washed once as described above.

Jelly was removed by centrifuging a concentrated egg suspension in an angle
centrifuge at approx. 7000 × g, after which the jelly layer could be removed by
suction. Alternatively, 0.15 N-HCl in sea water was added to a 20% suspension of
eggs in sea water, the volume ratio of HCl in sea water to egg suspension being 1 : 10.

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and Embryology.
After 5 min., during which the suspensions were occasionally agitated, the eggs were
washed twice with 60 vol. of sea water.

More than 95 % of the eggs prepared by these methods showed normal membrane
elevation after fertilization. In a few cases fertilized eggs were observed up to the
first cleavage stage. In these, 80-90 % of the eggs underwent normal cleavage.

For the preparation of homogenates or egg extracts, eggs packed by centrifugation
(angle centrifuge, 7000 x g) were suspended in an equal volume of M/50-phosphate
buffer, pH 7.5, which caused complete cytolysis. Extracts were prepared by centri-
fugation of homogenates, the opalescent supernatant being used as the enzyme
preparation.

Experiments were carried out in Thunberg tubes, the enzyme being placed in the
side-arms. The tubes were evacuated to 7 cm. Hg and filled with N₂ which had
previously been passed over hot Cu. After six cycles of evacuation and filling with
N₂, the tubes were placed in a water-bath at 20°C. and, after temperature equilibra-
tion, the contents mixed by tipping. One ml. of enzyme preparation, the extract
equivalent of 0.5 ml. eggs, was used in a final volume of 2-2.5 ml.

Lactic acid was estimated by the method of Barker & Summerson (1941), and
pyruvic acid by that of Friedmann & Haugen (1943).

The substances added to the egg extracts (forming 'complemented extracts')
were from the following sources: glycogen (rabbit liver), BDH; soluble starch,
Hopkins & Williams' Analar; cozymase (DPN), purity 21 % by the spectrophoto-
metric method of LePage (1949), Light and Co.; adenylic acid, Light and Co.;
adenosinetriphosphate (ATP), 60 % pure on P₁, P total and N₂ analysis, the contami-
ant being inorganic, Light and Co.; hexose diphosphate, Ca salt of 85 %
purity by easily hydrolysable and total P analysis, Light and Co.

Because of the sensitivity of sea-urchin egg homogenates to Ca (Hultin, 1950),
the hexose diphosphate was converted to the Ba salt by three cycles of solution
in N/5-HCl and precipitation at pH 8 in the presence of a large excess of Ba and
0.15 ml. ethanol. The resulting product was 95 % pure on easily hydrolysable and
total P analysis.

Analytically pure glucose-1-phosphate was prepared by the method of Sumner &
Somers (1944). Fructose-6-phosphate was prepared from hexose diphosphate by
that of Neuberg, Lustig & Rothenberg (1943). It was 97 % pure on P total and
fructose analysis. We are indebted to Prof. F. Dickens, F.R.S., for gifts of glucose-6-
phosphate and phosphogluconic acid (purity > 95 %) and to Prof. E. Baldwin for
a gift of phosphoglyceric acid (purity > 95 %).

All phosphorylated intermediates were in the form of Ba salts. The appropriate
weight of each was dissolved in N/5-HCl and the Ba precipitated with slightly more
than the theoretical quantity of Na₂SO₄. The BaSO₄ precipitate was washed once
with N/5-HCl, the combined supernatants brought to pH 7.5 and diluted to a final
concentration of m/20. All other solutions were brought to pH 7.5 before use.

Glycogen was estimated in jelly-free eggs by the alkaline hydrolysis and precipita-
tion methods of Lindberg (1945). After 30 min. hydrolysis in 5N-H₂SO₄ (see
Sahyun, 1933) and neutralization to litmus, the reducing sugar was determined
The metabolism of the sea-urchin egg

by the method of Folin & Malmros (1929). Total lipid was estimated by extracting packed eggs three times with 7% trichloroacetic acid (TCA) and washing with 0.5% TCA. The residue was treated once with 8 vol. cold ethanol, once with boiling absolute ethanol under reflux and once with a 3:1 ethanol-ether mixture under reflux. The extracts were evaporated to dryness, extracted three times with boiling chloroform, filtered and the dry weight of the chloroform extract determined. Aliquots of the chloroform extract were used for estimation of total P to give the phospholipid content.

RESULTS

Egg substrates. A sample of jelly-free eggs contained 3.6 mg. glycogen per ml. packed eggs. The dry weight of 1 ml. of packed eggs being 245 mg., the glycogen content was 1.5% of the dry weight. A sample of 20 ml. packed eggs contained 1.02 g. lipid, corresponding to a lipid content of 20% of the dry weight. 24% of the total lipid was phospholipid, on the assumption that the P-content of phospholipid is 4%.

Lactic acid production by whole eggs. After an induction period of about 1 hr., whole eggs produce lactic acid under anaerobic conditions (Fig. 1). No appreciable amounts of pyruvic acid are produced in these circumstances, while there is no lactic acid accumulation in the presence of oxygen. The induction period for anaerobic lactic acid production is not caused by the presence of small amounts of oxygen as it occurs in egg suspensions which have been subjected to prolonged gassing with N₂ passed over heated Cu. When the eggs begin to produce lactic acid in large amounts, microscopically visible signs of injury, in the form of cytolysed eggs, occur; the fertilizability of eggs is lost before cytolysis. By the time lactic acid production begins to decline, there is marked evidence of cytolysis.

Lactic acid production is inhibited by fluoride, iodoacetate, and phenylmercuric nitrate (Table 1). The plasma membrane may not be equally permeable (or impermeable) to each of these inhibitors, a factor which probably has an important influence on the results obtained with different inhibitors (cf. Table 4).

Table 1. Effect of inhibitors on acid production of whole eggs (Echinus esculentus)

<table>
<thead>
<tr>
<th>Inhibitor Concentration...</th>
<th>Lactic acid (μg.)</th>
<th>Pyruvic acid (μg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>300</td>
<td>3</td>
</tr>
<tr>
<td>Iodoacetate 10⁻⁴M</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>Phenylmercuric nitrate 10⁻⁴M</td>
<td>248</td>
<td>7</td>
</tr>
<tr>
<td>Fluoride 10⁻⁴M</td>
<td>121</td>
<td>0</td>
</tr>
</tbody>
</table>

Eggs in M/50-phosphate buffer produced more acid than those in M/50-phosphate buffer in 0.3 M-KCl, while the supernatants from homogenates were more active than whole homogenates.

Stimulation of glycolysis by cofactor addition. The sea-urchin egg glycolytic system is stimulated by the addition of cofactors (Table 2). Mg²⁺ did not stimulate extract glycolysis.
**Effect of different substrates on acid production.** The experiment in Table 3 indicates that lactic and pyruvic acid production is increased by the addition of fructose and glucose, and to a lesser extent, by glycogen and starch.

![Graph](image)

**Fig. 1.** Lactic acid production (µg./ml. eggs) in different samples of unfertilized eggs of *E. aculentus.* Curves I–IV refer to eggs kept at 20–21° C. during the experiment. The eggs in curve V were kept at 15° C. during the experiment and showed signs of injury during the third hour. The eggs in curve II were 3 hr. old before use and showed signs of injury after 1 hr.

**Inhibitors.** Inhibitors of glycolysis cause a marked inhibition of extract glycolysis (Table 4).

**Effect of phosphorylated intermediates on glycolysis.** The experiments in Table 5 show that the phosphorylated intermediates of the classical glycolytic system are also capable of yielding lactic and pyruvic acids in the sea-urchin egg system. The enzyme preparation used in the second experiment in Table 5 was made from eggs which had been left standing for 3 hr. before cytolysis. The experiment shows that under these conditions, the enzymes before aldolase, particularly phosphoglucomutase and
Table 2. Effect of cofactor addition on acid production

Each Thunberg tube contained M/20-phosphate buffer, pH 7.5 (final concentration). The additions were: glycogen, 12 mg.; cozymase (DPN), 200 μg.; ATP, 1 mg.; adenylic acid (AA), 1 mg. Exp. I, 1 ml of 50% extract, final volume, 2 ml. Exp. II, 1 ml of 30% extract, final volume, 2 ml. Incubation time, 60 min.

| System | Experiment I | | Experiment II | | |
|--------|--------------|--------|--------------|--------|
|        | Lactic acid (μg.) | Pyruvic acid (μg.) | Sum (μg.) | Lactic acid (μg.) | Pyruvic acid (μg.) | Sum (μg.) |
| (1) Extract | -8 | 55 | 47 | 0 | 5 | 5 |
| (2) (1) + glycogen | 28 | 139 | 147 | 3 | 8 | 11 |
| (3) (2) + DPN | 244 | 14 | 258 | 44 | 27 | 71 |
| (4) (2) + ATP | 66 | 147 | 213 | 7 | 6 | 13 |
| (5) (3) + ATP | 252 | 34 | 286 | 59 | 67 | 126 |
| (6) (5) + AA | 220 | 34 | 244 | 40 | 67 | 107 |
| (7) (5) without glycogen | 193 | 17 | 210 | 40 | 45 | 85 |
| (8) (2) + AA | - | - | - | 40 | 25 | 65 |

Table 3. Comparison of acid production with different substrates

Each Thunberg tube contained 1 ml of 50% extract, 200 μg. DPN, 1 mg. ATP, M/20-phosphate buffer, pH 7.5 (final concentration), final volume, 2 ml. Substrate concentration, M/40 (or M/40 glucose equivalent in the case of starch and glycogen). Incubation time, 60 min.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Nil</th>
<th>Glycogen</th>
<th>Starch</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Glucose-1-phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid (μg.)</td>
<td>137</td>
<td>235</td>
<td>273</td>
<td>334</td>
<td>337</td>
<td>275</td>
</tr>
<tr>
<td>Pyruvic acid (μg.)</td>
<td>93</td>
<td>149</td>
<td>147</td>
<td>162</td>
<td>176</td>
<td>234</td>
</tr>
<tr>
<td>Sum (μg.)</td>
<td>230</td>
<td>384</td>
<td>420</td>
<td>496</td>
<td>513</td>
<td>509</td>
</tr>
</tbody>
</table>

Table 4. Effect of inhibitors on reconstructed glycolytic system

Each Thunberg tube contained 1 ml of 40% egg extract, M/20-phosphate buffer, pH 7.5 (final concentration), 200 μg. DPN, 1 mg. ATP, and 12 mg. soluble starch, final volume, 2 ml. Incubation time, 60 min.

<table>
<thead>
<tr>
<th>Inhibitor ......</th>
<th>Nil</th>
<th>Iodoacetate</th>
<th>Fluoride</th>
<th>Phloridzin</th>
<th>Phenylmercuric nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration...</td>
<td>—</td>
<td>10^-4M</td>
<td>10^-4M</td>
<td>10^-4M</td>
<td>10^-4M</td>
</tr>
<tr>
<td>Lactic acid, μg.</td>
<td>111</td>
<td>18</td>
<td>80</td>
<td>20</td>
<td>82</td>
</tr>
<tr>
<td>Pyruvic acid, μg.</td>
<td>78</td>
<td>4</td>
<td>5</td>
<td>-4</td>
<td>-36</td>
</tr>
<tr>
<td>Sum, μg.</td>
<td>189</td>
<td>22</td>
<td>85</td>
<td>16</td>
<td>46</td>
</tr>
</tbody>
</table>

hexose isomerase, enzymes shown to be present in sea-urchin egg extracts by Ycas (1950), are the most labile.

DISCUSSION

The glycogen content of the eggs of *E. esculentus* is lower than that of other species (Orström & Lindberg, 1940; Hutchens, Kelitch, Krahl & Clowes, 1942), though it is about the same as that of oyster eggs (Cleland, 1950b). Oyster eggs, however, have a much lower glycolytic rate than those of *E. esculentus* (Table 6). The total lipid content of the latter is of the same order as in other species (Hayes, 1938; Ohman,
1944), although the phospholipid concentration is lower. The lack of visible lipid inclusions in the egg of *E. esculentus* is interesting in view of the high concentration of these substances.

The experiments described in this paper show that whole eggs are capable of anaerobic lactic acid production, which confirms the findings of Perlzweig & Barron (1928) and Ycas (1950). The time course of this lactic acid production is complex, the curve being more or less sigmoid, with an induction period of about an hour at 20°C. (cf. Dickens & Greville, 1932, 1933), and a declining rate of production after 2-3 hr. of anaerobiosis. Cytolysis at this stage might account for the decline in lactate production.

Having excluded the possibility that residual oxygen can account for the induction period, the following explanations of this phenomenon are worth consideration:

1. There exists a labile or easily saturated mechanism which can degrade pyruvic or lactic acid. Such a mechanism has been described in oyster eggs by Cleland (1950b). In this material a system is present on the cytoplasmic granules which is capable of removing added pyruvate under anaerobic conditions. The effect is stimulated by CO₂ or bicarbonate.

2. An intermediate product of glycolysis, which is not present, or present in limiting amounts, under aerobic conditions, is slowly produced. If this suggestion is correct, aerobic and anaerobic metabolism may proceed through different pathways.

3. The egg injury which ultimately occurs under the conditions of the experiments may increase the rate of glycolysis.

Explanation (1) is unlikely to be correct because homogenates cannot degrade added pyruvate anaerobically. Explanation (2) is also unlikely to be correct since the possible limiting factors (inorganic P, ATP, and adenylic acid) have been found in other sea-urchin eggs. The third explanation is most likely to be the correct one. Damaged eggs often exhibit an increase in O₂ uptake (Rothschild, 1949; Runnström, 1949; Cleland, 1950a), while completely cytolysed eggs may have a higher rate of O₂ uptake than uninjured unfertilized eggs (Warburg, 1914). In these experiments, injury, as evidenced by cytology or loss of fertilizability, coincided with the onset of rapid lactic acid production and it is reasonable to expect a causal relationship between the two phenomena. This does not mean that no anaerobic lactate production occurs until the eggs are irreversibly damaged, but that injury causes an increased production. This distinction is quantitative rather than qualitative and, in the case of *Psammechinus miliaris*, the eggs are fertilizable after the maximum rate of anaerobic lactic acid production has been reached.

Acid production by egg extracts is stimulated by the usual cofactors of glycolysis, as is shown in Table 2. The slight inhibitory action of adenylic acid when added with ATP, and the lack of stimulation when it is used alone, may be due to it being present in adequate quantities in uncomplemented extracts, or to the presence of heavy metal impurities in the sample of adenylic acid used. The failure of added Mg to stimulate glycolysis does not imply that Mg is not a normal requirement of the system and that therefore the system is non-glycolytic, for the following reasons: first, because free Mg⁺⁺ was present both in the egg homogenates, the concentra-
Table 5. Acid production using phosphorylated intermediates as glycolytic substrates

Each Thunberg tube contained 1 ml. 45% extract, 200 μg. DPN, 1 mg. ATP, 1 mg. AA, M/20-phosphate buffer pH 7.5 (final concentration), final volume, 2 ml. Final substrate concentration, M/200. Incubation time, 45 min. in Exp. I, 60 min. in Exps. II and III.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Experiment I</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Lactic acid</td>
<td>Pyruvic</td>
<td>Sum</td>
<td>Excess</td>
<td>Lactic</td>
<td>Pyruvic</td>
<td>Sum</td>
<td>Excess</td>
<td>Lactic</td>
</tr>
<tr>
<td></td>
<td>(μg.)</td>
<td>acid</td>
<td>(μg.)</td>
<td>over</td>
<td>acid</td>
<td>acid</td>
<td>(μg.)</td>
<td>over</td>
<td>acid</td>
</tr>
<tr>
<td>Control</td>
<td>57</td>
<td>60</td>
<td>117</td>
<td>—</td>
<td>5</td>
<td>26</td>
<td>—</td>
<td>—</td>
<td>60</td>
</tr>
<tr>
<td>Glucose</td>
<td>121</td>
<td>90</td>
<td>211</td>
<td>94</td>
<td>23</td>
<td>8</td>
<td>36</td>
<td>10</td>
<td>143</td>
</tr>
<tr>
<td>Glucose-1-phosphate</td>
<td>101</td>
<td>139</td>
<td>240</td>
<td>123</td>
<td>33</td>
<td>7</td>
<td>40</td>
<td>14</td>
<td>122</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>95</td>
<td>157</td>
<td>252</td>
<td>135</td>
<td>55</td>
<td>26</td>
<td>81</td>
<td>55</td>
<td>136</td>
</tr>
<tr>
<td>Phosphogluconate</td>
<td>61</td>
<td>68</td>
<td>131</td>
<td>74</td>
<td>14</td>
<td>18</td>
<td>23</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>103</td>
<td>252</td>
<td>355</td>
<td>238</td>
<td>55</td>
<td>117</td>
<td>373</td>
<td>247</td>
<td>176</td>
</tr>
<tr>
<td>Hexosediphosphate</td>
<td>136</td>
<td>248</td>
<td>384</td>
<td>267</td>
<td>135</td>
<td>196</td>
<td>332</td>
<td>306</td>
<td>205</td>
</tr>
<tr>
<td>Phosphoglycerate</td>
<td>61</td>
<td>670</td>
<td>731</td>
<td>614</td>
<td>29</td>
<td>750</td>
<td>779</td>
<td>753</td>
<td>57</td>
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</table>

Table 6. Comparison of glycolysis in different invertebrate tissues

<table>
<thead>
<tr>
<th>Animal</th>
<th>Organ</th>
<th>Temp. (° C.)</th>
<th>System</th>
<th>Complement</th>
<th>Q*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cockroach</td>
<td>Muscle</td>
<td>25</td>
<td>Teased fibres</td>
<td>Cofactors and substrate</td>
<td>0.4</td>
<td>Barron &amp; Talmian, 1948</td>
</tr>
<tr>
<td>Grasshopper</td>
<td>Muscle</td>
<td>37</td>
<td>Homogenate</td>
<td>Cofactors and substrate</td>
<td>0.67</td>
<td>Humphrey, 1949</td>
</tr>
<tr>
<td>Oyster</td>
<td>Muscle</td>
<td>37</td>
<td>Homogenate</td>
<td>Cofactors and substrate</td>
<td>2.7</td>
<td>Humphrey &amp; Siggins, 1949</td>
</tr>
<tr>
<td>Oyster</td>
<td>Egg</td>
<td>25</td>
<td>Extract</td>
<td>Cofactors and substrate</td>
<td>2.0</td>
<td>Humphrey, 1944</td>
</tr>
<tr>
<td>Schistosoma mansoni</td>
<td>Whole worm</td>
<td>37</td>
<td>Entire</td>
<td>None</td>
<td>0.5</td>
<td>Cleland, 1950</td>
</tr>
<tr>
<td>Sea-urchin</td>
<td>Egg</td>
<td>20</td>
<td>Whole egg</td>
<td>None</td>
<td>2.5</td>
<td>Bueding, 1950</td>
</tr>
<tr>
<td>Sheep</td>
<td>Liver</td>
<td>37</td>
<td>Extract</td>
<td>Cofactors and substrate</td>
<td>1.7</td>
<td>Bueding, 1950</td>
</tr>
<tr>
<td>Sheep</td>
<td>Brain</td>
<td>37</td>
<td>Extract</td>
<td>Cofactors and substrate</td>
<td>2.0</td>
<td>This paper</td>
</tr>
<tr>
<td>Sheep</td>
<td>Embryo</td>
<td>37</td>
<td>Extract</td>
<td>Cofactors and substrate</td>
<td>1.7</td>
<td>This paper</td>
</tr>
<tr>
<td>Sheep</td>
<td>Embryo</td>
<td>37</td>
<td>Extract</td>
<td>Cofactors and substrate</td>
<td>2.0</td>
<td>Reiner, 1947</td>
</tr>
<tr>
<td>Sheep</td>
<td>Embryo</td>
<td>37</td>
<td>Extract</td>
<td>Cofactors and substrate</td>
<td>1.7</td>
<td>Reiner, 1947</td>
</tr>
</tbody>
</table>

* μl. lactic acid or lactic + pyruvic acid/hr./mg. dry weight. The figures are calculated from the most active preparations quoted.
tions of which were high, and in the small quantities of interstitial sea water; and secondly, because fluoride inhibited acid production.

The experiments in Table 2 show that in contrast to whole eggs, in which only lactic acid accumulates, complemented egg extracts produce both lactic and pyruvic acids. Furthermore, the lactate-pyruvate equilibrium varies in different experiments and the proportion of the two acids can be greatly affected by DPN addition. The accumulation of pyruvate as well as lactate is characteristic of glycolysis in extracts of invertebrate material (Humphrey, 1949); in oyster-egg extracts, pyruvic acid is virtually the only acid which accumulates (Cleland, 1950). It has not yet been shown, however, that lactic and pyruvic acid accumulate in intact invertebrate cells.

The production by the extracts of lactic and pyruvic acid from polysaccharides and monosaccharides suggests that phosphorylase and hexokinase are present; but a high amylase activity might account for these results, in which case the presence of phosphorylase would be in doubt. The inhibition by phloridzin and the fact that glucose-1-phosphate is an efficient substrate favour the view that phosphorylase is present.

The experiments with inhibitors of glycolysis also suggest that a typical glycolytic cycle operates in the sea-urchin egg. Iodoacetate and fluoride, in the usual concentrations, depress the acid production of whole eggs and complemented extracts; while phenylmercuric nitrate, a powerful —SH inhibitor, has a marked inhibitory action. Phloridzin, which inhibits the acid production that occurs during the cytolysis of these eggs (Rothschild, 1939), also inhibits lactic acid production in complemented extracts.* Lindberg & Ernster (1948) attach some importance to their failure to inhibit the oxidation of glucose by iodoacetate, and regard this as confirmation of their view that glycolysis is not the principal mechanism for carbohydrate breakdown in the sea-urchin egg.† The responses to inhibitors of invertebrate enzyme systems are known to differ from those of mammalian systems. For example, iodoacetate does not inhibit lactic acid production in some insects (Barron & Tahmisian, 1948; Humphrey & Siggins, 1949), although a typical effect occurs in oyster eggs (Cleland, 1950). In any case, the experiments recorded in this paper show that iodoacetate does inhibit sea-urchin egg glycolysis, even though it may not inhibit the oxidation of glucose.

Apart from the evidence already given, the fact that the phosphorylated intermediates of mammalian glycolysis undergo rapid breakdown to lactate and pyruvate in sea-urchin egg extracts constitutes further and cogent evidence for the operation of a normal glycolytic cycle in this system. The three experiments cited in Table 5 show that the glycolytic rates with different substrates may vary from preparation to preparation, which might be expected in a complex system some of whose component enzymes are known to be labile. The small activity of phosphogluconic acid is probably due to slight contamination with glucose-6-phosphate. Sea-urchin eggs,

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* It would be wrong to assume that the acid of injury, observed manometrically by Rothschild (1939), is lactic acid; it almost certainly is not.

† As these experiments were done in the presence of M/10 NaF, it is most unlikely that any glycolysis would have been observed.
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like all eggs so far studied, have a low phosphatase activity (Connors & Scheer, 1947; Krugelis, 1947; Cleland, 1950b). It is therefore unlikely that dephosphorylation of the phosphorylated intermediates played any part in their observed effect on glycolysis.

These considerations lead to the conclusion that the sea-urchin egg possesses a glycolytic system whose activity is comparable with that found in other invertebrate tissues. Table 6 enables such a comparison to be made, the values for the sea-urchin egg being similar to those reported for a number of invertebrate muscles.

SUMMARY

1. The eggs of Echinus esculentus contain about 1.5% glycogen and 20% lipid (dry weight).
2. Whole eggs produce lactic acid under anaerobic conditions, the maximum rate of production being 0.15 µl. lactate/hr./mg. eggs (dry weight) at 20°C.
3. Lactic acid production is inhibited by fluoride, iodoacetate and phenylmercuric nitrate.
4. The supernatant from centrifuged egg homogenates prepared in M/50-phosphate buffer, pH 7.5, ('extract'), was found to be a satisfactory glycolytic enzyme preparation.
5. The production of lactic and pyruvic acid by such extracts is greatly stimulated by the addition of glycogen, cozymase and adenosine triphosphate, ('complemented extracts').
6. Complemented extracts glycolyse glucose and fructose with greater facility than glycogen or starch. With glucose or fructose as substrate, the rate of formation of lactate and pyruvate may reach 1.7 µl./hr./mg. eggs (dry weight) at 20°C.
7. Complemented extract glycolysis is inhibited by fluoride, iodoacetate, phenylmercuric nitrate and phloridzin.
8. Active extract glycolysis occurs with the following glycolytic intermediates as substrates: glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, hexose diphosphate and phosphoglyceric acid.
9. It is concluded that the sea-urchin egg (E. esculentus) possesses a typical glycolytic system, whose activity is comparable with that found in other invertebrate tissues.

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REFERENCES


