STUDIES ON ENZYME ACTION.

XLV. LIPASE ACTIONS OF THE WHOLE TROUT AT DIFFERENT AGES.

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INTRODUCTION.

The lipase or ester-hydrolyzing actions of extracts of the whole rat at ages from 3 days before birth until 3 years 15 days, and of extracts of the whole mouse at ages from 6 days before birth until 1 year 261 days were presented in previous communications. The changes in the “pictures” of relative actions on ten esters of these extracts as the animals increased in age were given in some detail, and the absolute enzyme actions, which were also recorded, were shown to undergo characteristic changes.

At the suggestion of Dr. C. B. Davenport of the Carnegie Institution of Washington, Station for Experimental Evolution, a similar study was undertaken with trout, because here it was possible to begin with the eggs at a very early stage, and to follow the enzyme actions to the adult fish. The materials were obtained from the State Fish Hatcheries, Cold Spring Harbor, Long Island, N. Y., and the investigation was made possible because of the constant cooperation and helpfulness of Mr. Stanley C. Walters, Superintendent of the Hatcheries. 

3 Thanks are due the State Conservation Commission for permission to use the fish and eggs of the Hatcheries in this investigation, and Dr. C. B. Davenport for the use of laboratory space in the Station for Experimental Evolution where the greater part of the experimental work was done.

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Experimental Methods.

Rainbow trout were used in this investigation. In studying the eggs, it was found that different results were obtained for the ester-hydrolyzing actions if whole eggs, finely ground, were used instead of aqueous extracts. It was therefore necessary to study the ground solid materials in a number of cases. In other respects, the experimental methods were essentially the same as those used in the earlier investigations.

The eggs were ground with a weighed amount of sea sand, then either extracted overnight with a definite quantity of water, or portions weighed out to which water was added and the ester-hydrolyzing actions determined directly. The very small fish were treated similarly. The larger fish were passed twice through a meat grinder and then either extracted with water or weighed portions used directly for the enzyme tests. The materials extracted with water were allowed to stand at room temperature overnight, filtered through paper, and portions of the cloudy or turbid filtrates used for the tests. For the experiments with the solids, the requisite amounts of water were added to the different portions and the enzyme tests made directly on these. Toluene was present throughout the extractions and the tests. The mixtures were brought to pH 7.0. The conditions of testing were the same as those described previously; 15 cc. of solution or mixture, 3.4 milli-equivalents of each of the ten esters, 22 hours incubation at 37°-38°, titration with 0.1 normal sodium hydroxide solution with phenolphthalein as indicator; duplicate and blank determinations.

In a number of cases during the summer months, the eggs were removed from the fish after killing the latter. Most of the tests,

--- Original solids. --- Aqueous extracts.

Fig. 1. Relative lipase actions of trout eggs and whole trout, original solids and aqueous extracts.

Chart a. Eggs, 5 hours after being taken. Chart b. Eggs, 35 days after being fertilized. Chart c. Fish, 2 weeks after being hatched. Chart d. Fish, 2 weeks after being fed. Chart e. Fish, 3 months after being fed. Chart f. Fish, 4 to 5 years old.

The difference in the two curves (original solids and aqueous extracts) are quite marked in Chart a, less so in Chart b, and still less so in Chart c. The two curves practically coincide in each of the last three charts.
however, were made with eggs taken for hatching purposes in November and later. These eggs were fertilized and kept in cold running water at the Hatchery in the usual way. They hatched after about 50 days, and began eating supplied food about 3 weeks after hatching. The ages of the older fish were only approximated.

EXPERIMENTAL RESULTS.

The relative actions on the ten esters of extracts and of original solids of the same materials are shown in Fig. 1. Six specimens at different stages of the life cycle were chosen, and each chart in the figure refers to one of these ages. It is evident that the “pictures” of the actions for the eggs at the earliest age chosen (Chart a) were entirely different for the extracts and the solids. The “pictures” differed considerably from each other at the next age (Chart b), but only to a small extent for the fish 2 weeks after hatching (Chart c). For the fish 2 weeks after feeding to the oldest studied, the curves were practically identical (Charts d, e, and f).

The eggs, for which the results are given in Fig. 1, were taken for hatching purposes. The absolute actions of the extracts of these eggs, some of which will be presented later, were comparatively small. The extracts of a number of sets of eggs removed after killing the fish, gave considerably larger absolute actions but essentially the same curves as the extracts of the more mature (but less soluble) eggs.

A considerable number of results was obtained with eggs and fish of different ages. In place of giving all of these results, a limited number are presented in Fig. 2. Since it is probable that where


TABLE I.

Hydrolyzing Actions in Tenths of Milli-Equivalents of Acid Produced by Aqueous Extracts and Whole Solids of Trout Eggs and Whole Trout of Different Ages on the Indicated Esters.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>PROAc</th>
<th>G3G0Ac (g)</th>
<th>MeG0Ac</th>
<th>CMeG0Ac</th>
<th>E0Ac</th>
<th>MeAc</th>
<th>CMeAc</th>
<th>E0Ac</th>
<th>MeAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs removed from fish</td>
<td>3.40</td>
<td>2.83</td>
<td>0.18</td>
<td>0.68</td>
<td>0.80</td>
<td>0.23</td>
<td>0.02</td>
<td>0.00</td>
<td>0.36</td>
</tr>
<tr>
<td>Eggs 4 days after fertilization</td>
<td>0.45</td>
<td>0.26</td>
<td>0.00</td>
<td>0.04</td>
<td>0.04</td>
<td>0.09</td>
<td>0.00</td>
<td>0.00</td>
<td>0.04</td>
</tr>
<tr>
<td>Eggs 37 days after fertilization</td>
<td>0.39</td>
<td>0.31</td>
<td>0.02</td>
<td>0.07</td>
<td>0.06</td>
<td>0.09</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Fish, 2 weeks after hatching</td>
<td>0.72</td>
<td>0.78</td>
<td>0.10</td>
<td>0.14</td>
<td>0.05</td>
<td>0.11</td>
<td>0.21</td>
<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>Fish, 1 week after feeding</td>
<td>1.03</td>
<td>1.31</td>
<td>0.28</td>
<td>0.35</td>
<td>0.19</td>
<td>0.26</td>
<td>0.29</td>
<td>0.05</td>
<td>0.34</td>
</tr>
<tr>
<td>Fish, 2 weeks after feeding</td>
<td>3.14</td>
<td>2.05</td>
<td>1.08</td>
<td>0.64</td>
<td>0.48</td>
<td>0.54</td>
<td>0.84</td>
<td>0.26</td>
<td>0.68</td>
</tr>
<tr>
<td>Fish, 3 weeks after feeding</td>
<td>1.66</td>
<td>1.62</td>
<td>0.69</td>
<td>0.43</td>
<td>0.33</td>
<td>0.40</td>
<td>0.64</td>
<td>0.14</td>
<td>0.46</td>
</tr>
<tr>
<td>Fish, 3 months after feeding</td>
<td>3.48</td>
<td>1.16</td>
<td>0.66</td>
<td>0.62</td>
<td>0.60</td>
<td>0.62</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish, 1 month after feeding</td>
<td>3.82</td>
<td>1.64</td>
<td>0.84</td>
<td>0.52</td>
<td>0.45</td>
<td>0.36</td>
<td>0.40</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>Fish, 2 months after feeding</td>
<td>3.62</td>
<td>0.84</td>
<td>0.52</td>
<td>0.45</td>
<td>0.36</td>
<td>0.40</td>
<td>0.43</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>Fish, 3 months after feeding</td>
<td>2.77</td>
<td>0.85</td>
<td>0.43</td>
<td>0.40</td>
<td>0.36</td>
<td>0.38</td>
<td>0.47</td>
<td>0.16</td>
<td>0.06</td>
</tr>
<tr>
<td>Fish, 6 months old</td>
<td>2.16</td>
<td>0.87</td>
<td>0.56</td>
<td>0.51</td>
<td>0.38</td>
<td>0.46</td>
<td>0.49</td>
<td>0.18</td>
<td>0.11</td>
</tr>
<tr>
<td>Fish, 1½ years old</td>
<td>0.73</td>
<td>0.51</td>
<td>0.26</td>
<td>0.25</td>
<td>0.18</td>
<td>0.45</td>
<td>0.24</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>Fish, 2 years old</td>
<td>1.21</td>
<td>0.43</td>
<td>0.25</td>
<td>0.30</td>
<td>0.21</td>
<td>0.29</td>
<td></td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td>Fish, 2½ years old</td>
<td>1.05</td>
<td>0.64</td>
<td>0.20</td>
<td>0.20</td>
<td>0.15</td>
<td>0.25</td>
<td>0.24</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>Fish, 4 to 5 years old</td>
<td>1.25</td>
<td>0.96</td>
<td>0.32</td>
<td>0.23</td>
<td>0.15</td>
<td>0.18</td>
<td>0.14</td>
<td>0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solids</th>
<th>PROAc</th>
<th>G3G0Ac (g)</th>
<th>MeG0Ac</th>
<th>CMeG0Ac</th>
<th>E0Ac</th>
<th>MeAc</th>
<th>CMeAc</th>
<th>E0Ac</th>
<th>MeAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs, 5 hours after taking</td>
<td>1.66</td>
<td>2.02</td>
<td>0.06</td>
<td>0.76</td>
<td>1.03</td>
<td>1.00</td>
<td>0.23</td>
<td>0.00</td>
<td>1.17</td>
</tr>
<tr>
<td>Eggs, 15 days after fertilization</td>
<td>1.51</td>
<td>1.88</td>
<td>0.01</td>
<td>0.70</td>
<td>1.09</td>
<td>1.02</td>
<td>0.22</td>
<td>0.00</td>
<td>1.08</td>
</tr>
<tr>
<td>Eggs, 50 days after fertilization (just hatching)</td>
<td>2.09</td>
<td>2.07</td>
<td>0.26</td>
<td>0.66</td>
<td>0.74</td>
<td>0.50</td>
<td></td>
<td>0.00</td>
<td>0.85</td>
</tr>
<tr>
<td>Fish, 2 weeks after hatching</td>
<td>2.97</td>
<td>3.09</td>
<td>0.65</td>
<td>1.13</td>
<td>0.94</td>
<td>1.11</td>
<td>0.78</td>
<td>0.14</td>
<td>1.34</td>
</tr>
<tr>
<td>Fish, 3 weeks after feeding</td>
<td>3.38</td>
<td>2.48</td>
<td>1.39</td>
<td>0.88</td>
<td>0.81</td>
<td>0.77</td>
<td>1.06</td>
<td>0.33</td>
<td>2.09</td>
</tr>
<tr>
<td>Fish, 3 months after feeding</td>
<td>3.95</td>
<td>1.27</td>
<td>0.81</td>
<td>0.67</td>
<td>0.54</td>
<td>0.48</td>
<td>0.71</td>
<td>0.33</td>
<td>0.25</td>
</tr>
<tr>
<td>Fish, 4 to 5 years old</td>
<td>2.52</td>
<td>1.79</td>
<td>0.61</td>
<td>0.62</td>
<td>0.45</td>
<td>0.50</td>
<td>0.42</td>
<td>0.04</td>
<td>0.07</td>
</tr>
</tbody>
</table>
differences were found in the curves obtained with the solids and the
extracts, the significant results are those obtained with the former,
the results are shown for the eggs and the youngest fish in the first
three charts for the ester-hydrolyzing actions of the solid materials,
and in the last three charts for the extracts which are essentially the
same as the results for the solids. Chart c for the solids and Chart d
for the extracts refer to the same materials. The changes in the
"pictures" of the actions of the youngest eggs up to the time that the
young fish began to eat are quite clear. Thereafter the changes are
not so distinct although some trends are discernible.

Absolute values of the ester-hydrolyzing actions of a number of the
materials are shown in Table I. The results for extracts and whole
solids are given for different ages of the eggs and fish. Not all the
results which have been obtained are presented, but the data may be
considered to be representative of the various actions. The concen-
trations in all cases refer to 44.4 mg. of original material per cc. of
final solution or mixture tested.

Although the results presented in Table I are somewhat irregular,
certain definite conclusions may be drawn from them. The extracts
of the eggs removed from the fish after killing the latter (before
October 1st) gave much larger actions than did the extracts of the eggs
taken for hatching (after November 1st). Extracts of these more
mature eggs gave only very small actions after fertilization as well as
before. After hatching, the actions began to increase, markedly so
2 or 3 weeks after feeding, although some irregularities are apparent
at this time, and apparently reached maximum values 1 to 2 months
after feeding. For the older fish the actions decreased again.

With the whole solids, the absolute actions increased starting with
the eggs attaining comparatively large values for the young fish.

Without going into the details of the absolute actions on all of the
individual esters, certain facts of interest may be mentioned. With
both extracts and solids, the actions of the eggs on methyl and ethyl
butyrates were practically zero. Especially for the solids, the large
actions on isobutyl acetate in comparison with the very small actions
on ethyl butyrate (its isomer) are striking. After the fish had begun
to feed, the actions on the butyrates increased markedly, to decrease
again as the fish became older. In general terms, the acetic esters
were hydrolyzed to considerable extents, although here there were considerable variations with the different alcohol radicals. The higher actions on glyceryl triacetate in comparison with those on phenyl acetate for the solids (and to a less extent for the extracts) with the eggs and youngest fish may be indicated. Other regularities might be pointed out but would not add anything of significance to the findings.

**DISCUSSION.**

In planning this investigation, it was hoped that the methods of enzyme study applied to the life cycles of whole rats and whole mice would be of interest with fish where it would be possible to procure eggs at a very early stage. The experimental study was complicated by the fact that with the eggs, solubility influences played a part, so that the “pictures” or curves of the relative ester-hydrolyzing actions were different for the extracts and whole solids. These differences did not exist with the fish after a short period of feeding. The changes in the “pictures” of enzyme actions from the eggs (solids) to the fish are quite clear, but there appeared to be no further changes of any degree of definiteness as the fish became larger and more mature. After the fish had eaten supplied food for several weeks the type of actions appeared to remain unchanged. Another fact which may have wider significance relates to the comparatively large solubility of the immature eggs in comparison with the insolubility (as far as substances carrying the ester-hydrolyzing actions are concerned) of the more mature eggs taken for purposes of hatching, although the types of the actions were the same for the two.

**SUMMARY.**

The ester-hydrolyzing or lipase actions of extracts and whole solids of trout eggs and whole trout of different ages were tested on ten simple esters by the method described in previous papers. Differences in solubility of the enzyme materials of the eggs were found. The “pictures” of the relative enzyme actions changed from a type found with immature eggs to a type which became constant for the fish after they had eaten for 2 weeks. After this, the type did not change up to the age of 4 to 5 years (the oldest trout studied). The absolute ester-hydrolyzing actions of the materials were also presented and discussed.