Catalase and Aldolase in Livers of Regenerating and Tumor-bearing *Triturus viridescens*

Morton Rothbard†

(Biology Department, University of Notre Dame, Notre Dame, Ind.)

The urodele, *Triturus viridescens*, is able to regenerate all four of its limbs following amputation and can also support the growth of a fibrosarcoma (4). It is therefore unique in that it is the highest vertebrate in which true epimorphic regeneration can occur on a large scale and into which a relatively active tumor can be transplanted. The adult newt was investigated by Breedis (4), who chemically induced the formation of both accessory limbs and tumor. Rubens (20) obtained supernumerary limb production in newts by implanting the anuran Lucké renal carcinoma.

Brahn (3), Greenstein (9), Begg et al. (2), Hargreaves and Deutsch (12), Nakahara and Fukuoka (17), and Lucké and Berwick (15), working with a variety of tumor products, found a decreased liver catalase activity in tumor-bearing mammals. However, Klett and Taylor (14) relate liver catalase alterations to changes in liver weight; Appleman (1) found that a lowering of liver catalase activity can also be caused by inanition.

Like catalase, aldolase has been shown to be altered in tissues of tumor-bearing animals (21, 25). Liver aldolase was shown to be lowered and serum aldolase to be raised in tumor-bearing animals. However, no changes in enzyme activity resulted from pregnancy or liver regeneration. Although aldolase is one of the few glycolytic enzymes affected by tumorigenesis, this aspect has received relatively little attention. Needham (18) indicates the need for more information concerning the metabolic relations between these two tissues (tumors and regenerates), since it has been shown that similarities exist (viz., histogenesis and metabolism). It was the object of this work to study liver aldolase and catalase activities in tumor-bearing and regenerate-bearing animals to determine whether these growths would elicit similar reactions in these enzymes.

* This work was supported in part by Public Health Service Fellowship No. CF 6039.

† Present address: St. Louis University Medical School, St. Louis, Missouri.

Received for publication September 5, 1958.

MATERIALS AND METHODS

Non-strain-standardized *Triturus viridescens* were maintained in aquaria at 30° C. The tumors proliferate at this temperature, but atrophy at room temperature.

The liver was removed immediately after weighing the animal and was homogenized in a Potter homogenizer. Time intervals between excision and homogenization (approximately 90 minutes) were standardized to eliminate any errors due to drying of the liver. Each liver was homogenized in 44 times its weight of cold (5° C.) distilled water and kept near 0° C. until used. Two water dilutions of the homogenate were made, 1:10 and 1:25. All homogenizations were done at 1,800 r.p.m. for 1 minute.

Animals with transplanted tumors derived from a tumor originally induced by methylcholanthrene were initially obtained from Dr. Charles Breedis. Transplants were made by injecting a tumor tissue brei (in physiological salt solution) into normal animals. Eighty per cent of the injected animals developed cancers. The number of takes was not correlated with the age of the tumor. However, necrotic cancers transplant poorly.

Limb amputation of anesthetized animals was performed in an ultraviolet-equipped box. Each limb was severed just proximal to the first limb joint. Locomotion of the "limbless" animals was not impaired, since newts use their tails for movement in water.

Catalase was determined according to the method of Feinstein (7). All activities were expressed as milliequivalents of perborate used per milligram of liver tissue in 5 minutes. Nitrogen determinations were made on a few samples to determine whether there was a change of liver weight without a corresponding activity change. No such complication was found.

Aldolase activity was determined by the method of Sibley and Lehninger (22) using a hexose diphosphate (HDP) molarity of 0.015 and a 37° C. water bath for incubation. The color complex formed in this method was read in a Fisher Electrophotometer with a 540 mμ filter. The method of Roe (19) was used for the determination of HDP molarity, and that of Fiske and Subbarow (8) for the determination of phosphorus.

RESULTS

Catalase

Since in preliminary experiments higher activities were usually obtained when homogenization motor speeds and time were approximately 1800 r.p.m. and 1 minute, respectively, these constants were used throughout.

Catalase assay of normal newts.—Because of

1 *Triturus viridescens* were obtained from J. C. Nicholls, Murphy, North Carolina.

A 1 per cent solution of tricaine methanesulfonate (MS 222) was used for anesthesia. MS 222 was obtained from Sandoz Chemical Works, Inc., 6878 Charleston St., New York City.
widely differing parameters among the newt liver catalase activities, it was necessary to perform many tests for each experiment. The results from more than 50 assays gave a control mean catalase activity of 0.167 for *T. viridescens*.

To ascertain whether the ratio of the size of the liver to total body weight would affect the amount of catalase in liver, a correlation test was done. The liver weight was directly correlated with the animal weight with a correlation coefficient of 0.90, but the total amount of liver catalase was independent of the size of the liver relative to that of the body. There was no correlation between the size of the animal and its liver catalase activity.

**TABLE 1**

**LIVER CATALASE* ACTIVITY OF Triturus**

<table>
<thead>
<tr>
<th>Liver source</th>
<th>No. samples</th>
<th>Max.</th>
<th>Min.</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>50</td>
<td>0.208</td>
<td>0.074</td>
<td>0.167</td>
</tr>
<tr>
<td>Tumor-bearing</td>
<td>19</td>
<td>0.415</td>
<td>0.197</td>
<td>0.299</td>
</tr>
<tr>
<td>Regenerating</td>
<td>17</td>
<td>0.468</td>
<td>0.121</td>
<td>0.302†</td>
</tr>
</tbody>
</table>

* Activity = m.eq. of perborate used/mg of tissue/15 min.
† Average of seven animals sacrificed during the 13th and 14th day of regeneration. Results from the first 12 days and the last 15 days are similar to those obtained for normal animals.

A correlation test was done to determine whether there was any relation between the ratio of tumor weight to body weight and the liver catalase activity. Data on tumors ranging from 10 per cent to more than 30 per cent of the total body weight revealed no such relationship (*t* = 0.075). If liver and cancer weights are compared, a correlation of 0.80 is obtained. Thus, since liver weight is directly proportional to body weight, a larger animal will be host to a larger tumor.

Catalase assay of regenerating newts.—In this experiment, the effects of regeneration on liver catalase activity were studied from the first to the 33d day following the amputation of all four limbs. No significant change occurred during the first 12 days, but a sharp increase in liver catalase activity was noted on the 13th and 14th days (Table 1). From the 14th to the 17th day there was a decline in activity to normal, where it remained until the end of the experiment. A significance test showed that the observed differences between mean liver catalase activity of the 13th day regenerates and that of the other two groups and control animals were real. There was no significant difference between the high liver catalase values of the cancerous animals and those of the regenerating animals.

**ALDOLASE**

Since the chemistry of the chromogen reaction in the assay for aldolase is not fully understood (22), the presence of aldolase in livers of *T. viridescens* had to be proved. Also, there are two well known pathways of glycolytic breakdown, only one of which requires aldolase. Consequently, it was necessary to show that a direct quantitative and linear relationship existed between the chromogen readings and the amount of alkali-labile phosphorus found after completion of the aldolase assay. An accumulation of alkali-labile P was found when extracts of liver were prepared and incubated with hexose diphosphate at 37° C. and pH 8.6, and the amount of alkali-labile P in the reaction mixture was proportional to the amount of enzyme extract used. Since the chromogen read-
ings were also proportional to the amount of extract used, linear relationship existed between the chromogen readings and the alkali-labile phosphorus released, and aldolase could be assayed in newt livers by the method of Sibley and Lehninger.

**Aldolase assay of normal newts.**—All aldolase values are given as micromoles of alkali-labile P released per gram of liver tissue per hour. The individual aldolase values for control animals varied widely from the average of 5.2. This necessitated the use of large numbers of animals.

Liver aldolase content was not significantly different between sexes ($r = 1.03$), nor was it correlated with salamander weight ($r = 0.42$). Also, the ratio of the weight of the tumor to the weight of the animal was not correlated with the liver aldolase amount ($r = 0.23$).

**Aldolase assay of tumor-bearing newts.**—Fifty-seven cancerous animals were used in this experiment. The observed difference between the mean liver aldolase amount of tumor-bearing newts (3.9) and that of the control group (5.2) was significant. The values for the two groups overlapped, but this was not surprising in view of the high degree of variation even within a control group.

Sibley and Lehninger (22) found that the amount of liver aldolase in tumor-bearing rats was one half the normal value.

**Aldolase assay of regenerating newts.**—Of 37 animals, eighteen controls and nineteen 1–12-day regenerates, there appeared to be slight significance between the average value for the normal group of 5.2 and that of the regenerating group of 5.9. After this period of high aldolase content, there was a decline so that the livers of regenerating animals, assayed from the 14th to the 21st day after amputation, gave an average value of 4.2. The apparent difference between the means for the controls and regenerates was also significant (Table 2).

The difference between the means of the tumor-bearing animals and the 1–12-day regenerates was real ($t = 4.01$), whereas that between the tumor-bearing animals and 14–21-day regenerating newts was not ($t = 0.38$).

### DISCUSSION

The hypothesis that a regenerating blastema biochemically resembles malignant tissue is supported by this study and those of other investigators. That both tumors and new limbs may arise from identical conditions is well established (4); both supernumerary limbs and tumors were produced from identical injections of methylcholanthrene. Rubens (20) noted that an implanted anuran cancer in a urodele limb can also cause production of supernumerary limbs. In view of such phenomena, the data reported in this paper are not surprising.

Chalkley (6) found that all the tissue types in the limb blastema of *T. viridescens* have their highest mitotic index at 13 days after amputation. This peak is reached by a sharp rise from the 7th day to the 13th day, after which there is a gradual decrease in the index to the 37th day. Thus, a relationship may exist between the liver catalase activity and the number of mitoses in the regenerating limb. The Breedis sarcoma, like many tumors, also contains many mitotic figures. In accord with this hypothesis is the work of Castiglioni-Pitotti (5) who determined the catalase activity of *Petromyzon* embryos. He found that catalase activity begins to increase with the neurula stage, reaching a maximum at the 18th day of development. Mistruzzi (16) found a higher catalase activity in the developing frog followed by a remarkable decrease at metamorphosis. In animals showing lowered liver catalase, it is of interest to note that Stein et al. (24) found such lowered activity in kidney at the period of highest mitotic activity of regenerating liver. It is felt that a large number of mitoses present both in regenerating limbs at 13 days post-amputation and in the Breedis sarcoma might be associated with higher level of liver catalase in the host.

The present study indicates that both tumorous and 14–21-day regenerating salamanders had lowered amounts of liver aldolase. This could indicate a shift to glycolysis from the Embden-Meyerhof chain of reactions to the Warburg-Dickens scheme. Results from experiments designed to test

### Table 2

**Liver Aldolase Activity of Control, Regenerating, and Tumor-Bearing Newts**

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of Samples</th>
<th>Max.</th>
<th>Min.*</th>
<th>Average*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-12-day regenerates</td>
<td>19</td>
<td>6.8</td>
<td>4.3</td>
<td>5.9</td>
</tr>
<tr>
<td>13-day regenerates</td>
<td>7</td>
<td>5.8</td>
<td>3.6</td>
<td>4.9</td>
</tr>
<tr>
<td>14-21-day regenerates</td>
<td>12</td>
<td>4.9</td>
<td>3.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>6.5</td>
<td>3.6</td>
<td>5.2</td>
</tr>
<tr>
<td>Tumor-bearing</td>
<td>27</td>
<td>4.2</td>
<td>2.1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

* Micromoles of alkali-labile P/hr/gm of liver. Assays were run at pH 8.6 and at 37° C.

"t" value for difference between the means of tumor-bearing and control liver aldolase values is 4.05.

The "t" values for the difference between the means of the regenerating and control values are:

- 1–12 days = 2.49
- 13 days = 1.48
- 14–21 days = 2.65

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this possibility by assaying for glucose-6-phosphate dehydrogenase will be published.

The use of *T. viridescens* as a tool toward acquiring greater knowledge of both the cancer and the regenerative processes is indicated. Both malignant and regenerating tissues belonging to the same animal can be studied concomitantly.

**SUMMARY**

1. The livers of normal, tumor-bearing, and regenerating (quadruple limb amputations) *T. viridescens* were assayed for catalase and aldolase activity.

2. A significant increase in liver catalase activity was found in both tumor-bearing and 13-day regenerate-bearing animals.

3. Changes in aldolase content were evident in both tumor-bearing and regenerating newt livers; they were low in the former and diminished in the latter group as time after amputation increased.

**ACKNOWLEDGMENTS**

The author wishes to express grateful acknowledgment to Dr. D. T. Chalkley of the National Institutes of Health for his assistance in formulating and directing the initial phase of the investigation; and to Dr. A. L. Schipper of Oak Ridge National Laboratory for editing the thesis manuscript.

I am particularly pleased to acknowledge an indebtedness to Dr. Charles Breedis of the University of Pennsylvania Medical School, who developed the original salamander tumor and who has supplied me with viable tumors and suggestions for its growth on several occasions.

**REFERENCES**


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