Dehydroascorbic acid, the oxidized form of ascorbic acid, is rapidly reduced to ascorbate in living organs (ascorbate recycling). We examined the GSH-dependent dehydroascorbate reductase activity in several tissues of the chicken. The activity was highest in the liver, and second highest in the brain. The activity was localized in the cytosol fraction of the liver. We subsequently examined the dehydroascorbate reduction in separated chicken hepatocytes. The cellular ascorbate concentration was elevated in dehydroascorbate-treated cells. It is thought that hepatocytes incorporated external dehydroascorbate and converted it into ascorbate. These findings suggest that the liver plays an important role in ascorbate recycling by the chicken.

Key words: dehydroascorbic acid; chicken; GSH-dependent dehydroascorbic acid reductase; hepatocytes

Ascorbic acid (AA) is also known as the important antioxidant, vitamin C, and is oxidized into monodehydroascorbic acid or dehydroascorbic acid (DAA). In mammals and highly evolved birds, AA is commonly synthesized from glucose in the liver, except for primates and a few other species. In primitive birds such as the chicken, the AA synthesis pathway is expressed in the kidney. Enzymatic DAA reduction into AA is considered important for chicken liver to maintain the amount of AA needed to protect against xenobiotics and oxidative stress.

In rats, a novel enzyme acting for GSH-dependent DAA reduction (EC 1.8.5.1) has been purified from liver cytosol. The recycling system in cultured human cells has also been described. However, little information on the DAA recycling system has been reported in chickens and other birds.

In the present study, we examined the DAA reduc- tase activities in several chicken tissues and investigated DAA reduction in primary cultured chicken hepatocytes.

Table 1 shows the GSH-dependent DAA reductase activities in several chicken tissues harvested from 16-day-old White Leghorn males. All animals received humane care as outlined in the Guide for the Care and Use of Experimental Animals (National Institute of Animal Industry Animal Care Committee). Tissue homogenates were centrifuged at 12,000 × g for 30 min and applied to the enzyme assay. The activity was determined as described by monitoring the changes in A265 associated with the formation of AA with 0.25 mM GSH and 1.0 mM DAA. One unit of DAA reductase activity is expressed as the amount that catalyzed the formation of 1 μmol of AA per min at 30°C.

The activity in the liver was higher than that in other tissues, and the brain had the second-highest activity. It has been reported that AA is needed to maintain the liver functions, e.g., xenobiotic metabolism. We considered that the enzymatic DAA reduction system would be one of the important mechanisms for maintaining an adequate amount of AA to ensure adequate function of the chicken liver which cannot maintain its AA level by

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific activity (10⁻¹ units/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>13.95 ± 4.04 (5)</td>
</tr>
<tr>
<td>Brain</td>
<td>9.14 ± 3.49 (5)</td>
</tr>
<tr>
<td>Heart</td>
<td>6.17 ± 1.63 (5)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>4.69 ± 1.65 (4)</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.44 ± 0.58 (5)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.43 ± 0.88 (4)</td>
</tr>
<tr>
<td>Lung</td>
<td>0.40 ± 0.23 (4)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE. The number of tissue samples is shown in parentheses.

Abbreviations: AA, ascorbic acid; DAA, dehydroascorbic acid; PDI, protein disulfide isomerase
Dehydroascorbate Reduction in Chicken

AA synthesis. Paolicci et al. have reported a high activity of GSH-dependent DAA reductase in rat liver, this finding agreeing with the results obtained in the present study.

Such activities were also investigated in subcellular fractions of the liver, and were only observed in the cytosol fraction (data not shown). Figure 1 shows AA formation from DAA (0.25 mM) by liver cytosol at several GSH concentrations. The activity increased with increasing GSH concentration and was not apparent without GSH. This finding suggests that GSH acted as a reductant in the enzymatic DAA reduction by liver cytosol. Our result agrees with the findings for rats. On the other hand, it has been reported that thioltransferase and protein disulfide isomerase (PDI) formed a GSH-dependent DAA reduction system. PDI, however, has been shown to a microsomal enzyme. Since the GSH-dependent DAA reductase activity was only found in the chicken cytosol fraction, it is considered that the enzyme might be independent of the thioltransferase/PDI system. Further study of the contribution of PDI to the enzymatic GSH-dependent DAA reduction system in the chicken is needed.

Figure 2 shows time-course changes in the intracellular reduced and total AA concentration of cultured chicken hepatocytes. Chicken hepatocytes were separated from 16-day-old White Leghorn male chickens and cultured as described. The plated cells were cultured with a serum-free L-15 medium containing 0.568 mM (equivalent to 100 mg/l of AA) DAA or AA. Cellular AA was determined by the α,α'-dipyridyl method as described.

As shown in Fig. 2A, cellular reduced AA was increased by both DAA and AA supplementation. Fig. 2B shows the time-course characteristics of the total cellular AA (AA plus DAA) concentration. In both DAA- and AA-treated hepatocytes, the total cellular AA level had increased 3 h after supplementation. Both intracellular reduced AA and total AA were unchanged in hepatocytes cultured with an AA- and DAA-free medium during this experimental period.

The L-15 medium did not contain AA. Therefore, the elevation of cellular reduced AA in the DAA-treated cells suggested that DAA was taken up and reduced to AA in the hepatocytes. This DAA transformation to AA that was observed in the hepatocytes suggests that GSH-dependent DAA reductase might have contributed to DAA reduction in the liver. The AA concentration in DAA-treated cells, however, was lower than that in AA-treated cells. DAA might have been broken down before its uptake by the hepatocytes, because DAA is unstable under neutral conditions.

The cellular amounts of both total and reduced AA decreased after the initial rise, however. We consider that cellular AA had been consumed by the hepatocytes themselves, or leaked into the medium after the initial rise, similar to what was found in an...

![Fig. 1. GSH-dependent Dehydroascorbate Reduction by Chicken Liver Cytosol](image-url)

Dehydroascorbate (0.25 mM) and liver cytosol were incubated with 0, 1.0, 2.0 and 5.0 mM GSH, and the formation of ascorbate monitored for 1 min at 30°C. The ascorbate formed without cytosol was also determined and subtracted.

![Fig. 2. Changes in the Cellular Reduced and Total Ascorbate Concentrations in Primarily Cultured Chicken Hepatocytes Treated with Dehydroascorbate or Ascorbate](image-url)

Hepatocytes were treated with 0.568 mM dehydroascorbate or ascorbate for 1, 3 and 6 h. (A) Reduced ascorbate concentration and (B) total (reduced plus oxidized-form) ascorbate concentration. ○ ○, control group (N=4); △ △, ascorbate treated (N=4); □ □, dehydroascorbate treated (N=3). Each value is expressed as the mean ± SD. Values with an asterisk mean a significant difference from the control group (p<0.05) as analyzed by Student's t-test.
investigation of a human liver cell line. To understand the mechanism, the AA and DAA transport systems in birds must be examined.

We found in the present study an enzymatic DAA reduction system in chicken for the first time and identified the liver as being the major tissue which expressed enzymatic DAA reduction. DAA reductase activity was found in the liver cytosolic fraction and was GSH-dependent. Indeed, it seems that the separated hepatocytes rapidly reduced external DAA. These findings suggest that the liver played an important role in the enzymatic AA recycling system in the chicken. In addition, enzymatic DAA reduction is considered to be important for maintaining the AA level and AA efficiency in chickens, in the same manner as that in mammals. Chicken liver lacks an AA biosynthesis system, so DAA reduction may be more important than in other tissues in order to maintain the AA level and such AA-related liver-specific functions as xenobiotic metabolism.

References