Acidic Peptides of the Lens

4. THE BIOSYNTHESIS OF OPHTHALMIC ACID*

BY E. E. CLIFFE AND S. G. WALEY

Nuffield Laboratory of Ophthalmology, University of Oxford

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Ophthalmic acid, a tripeptide isolated from calf lens (Waley, 1956, 1957), has been shown to have the structure \( I: R = CH_3 \) (Waley, 1958). This structure is very similar to that of glutathione (I: \( R = SH \)), and so the question naturally arises whether the two peptides are formed in vivo by the same route.

\[
\begin{align*}
H_2N\cdot CH(CO_2H)\cdot(CH_2)_2\cdot CO\cdot NH\cdot CH(CH_2)R\cdot & \\
CO\cdot NH\cdot CH_2\cdot CO_2H
\end{align*}
\]

(I)

The synthesis of glutathione in liver extracts takes place in two stages, each catalysed by a separate enzyme (Snoke & Bloch, 1952; Snoke, Yanari & Bloch, 1953; Yanari, Snoke & Bloch, 1953):

Glutamic acid + cysteine + ATP →

\[ \gamma \text{-glutamyl cysteine + ADP + H}_2\text{PO}_4 \]

\[ \gamma \text{-Glutamyl cysteine + glycine + ATP → glutathione + ADP + H}_2\text{PO}_4 \]

(ATP, adenosine triphosphate; ADP, adenosine diphosphate).

Lens extracts catalyse the synthesis of glutathione from the three amino acids and ATP (Daisley, 1955), presumably by the same two stages. This communication describes the synthesis of ophthalmic acid from its three amino acids and ATP in lens extracts; this synthesis also proceeds by a two-stage mechanism; moreover, at least one of the enzymes of the synthesis of glutathione also plays a part in the synthesis of ophthalmic acid.

EXPERIMENTAL

Materials

Lens preparations. Lenses were extracted from the eyes of calves within 2 hr. of the death of the animals; they could be stored in a frozen state for at least 4 weeks without impairment of enzymic activity. The lenses of rabbits (Dutch, 3-4 months old) were used fresh. The average weight of a calf lens is 1 g., and of a rabbit lens 0-35 g. Two types of lens preparation were used. In the first type (called whole lens), the lenses were ground at \( 4^\circ \) with an equal weight of water and a little sand; the mixture was not centrifuged. In the second type of preparation (called dialysed lens), this whole lens preparation was dialysed at

4\(^\circ\) for 24 hr. against water, and then for 24 hr. against 0-005 M -potassium phosphate, pH 6-4. The membrane was cellulose dialysis tubing, 0-75 in. in diameter (Gallenkamp and Co., London); the diffusate sacks were inverted, and the outer liquid renewed, every 2 hr. during the day. Thorough dialysis was necessary to remove most of the glutathione from the lens mixture. The contents of the dialysis sack were centrifuged for 20 min. at 4000 g at \( 4^\circ \), and the supernatant (1-7 ml. from 1 g. of lens) was used.

Peptides. Ophthalmic acid, norophthalmic acid, \( \gamma \)-L-glutamyl-L-\( \alpha \)-amino-n-butyr acid and L-\( \alpha \)-amino-n-butyrylglycine were prepared as previously described (Waley, 1958), and the glutathione was from The Distillers Co. Ltd.

Co-factors. The barium salt of n-3-phosphoglyceric acid (Light and Co., Colnbrook, Bucks.) was converted into the potassium salt with Amberlite IR-120(H\(^+\)) and KOH (Wade & Morgan, 1954).

Methods

The reactions were followed by paper electrophoresis. There are many advantages in following reactions catalysed by crude tissue extracts by an analytical method in which the products are separated and identified, and this is particularly important when several reactions are proceeding concurrently (cf. Waley & Watson, 1953, 1954). Paper electrophoresis was preferred to paper chromatography mainly because compact, intense spots with a low background colour were obtained by the procedure described below.

System used for synthesis of peptides. The concentrations of amino acids (or peptides) were 8 mm, except that the concentration of DL-\( \alpha \)-amino-n-butyric acid was 16 mm. The mixture also contained ATP (Sigma Chemical Co.) (2-5 mm), 3-phosphoglyceric acid (22 mm), KCl (42 mm), MgSO\(_4\) (10 mm) and potassium phosphate buffer, pH 6-4 (6 mm). The total volume of the solution was 10 ml., and it contained 7-7 ml. of calf-lens preparation (the experiments with rabbit lenses were done on a smaller scale); the reaction was carried out (at pH 6-3) at 37\(^\circ\) for 5 hr.; samples were withdrawn at intervals; after removal of protein and evaporation, the residues, in 10% (v/v) propan-2-ol, were examined by paper electrophoresis.

Removal of protein. Samples were taken at intervals from the reaction mixture and protein was precipitated by the addition of 2-5 vol. of hot ethanol; a measured fraction of the supernatant was evaporated in vacuo and the residue dissolved in 10% (v/v) propan-2-ol. The protein precipitate is bulky and estimations with the ninhydrin reagent of Troll & Cannan (1953) (after known amounts of ophthalmic acid had been added) showed that the amount of ophthalmic acid in the supernatant was only 87% of that expected, owing to adsorption on the protein precipitate. Hence this correction factor has been applied throughout.

Removal of glutathione. The electrophoretic mobility of reduced glutathione (GSH) is the same as that of oxothialmic acid, hence the necessity for the thorough dialysis described above. In some experiments in which GSH was necessarily present in the incubation mixture, the GSH was oxidized either to the disulphide (GSSG) or to the sulphoneic acid (GSO₄H). Autoxidation of the GSH to GSSG was effected at pH 8 by dissolving the residue (after removal of proteins) in 0.2 M-KHCO₃ and allowing the solution to stand in open tubes for 3 hr., with occasional agitation to ensure adequate access of oxygen. The electrophoretic mobility of GSSG is greater than that of GSH, as would be expected from the titration curves (Pirie & Pinhey, 1929). Oxidation of GSH to GSO₄H with performic acid was carried out as described previously (Waley, 1956); oxothialmic acid was found to be unaffected by performic acid.

Paper electrophoresis. A ridge-pole apparatus was used (Durrum, 1950); the apex height was 11.5 cm., and the paper sheets (Whatman no. 52) were 25 cm. long (measuring from one electrode to the other), and up to 44 cm. wide. The paper was soaked in a pyridine acetate buffer (50 ml. of acetic acid, 15 ml. of pyridine and 2.51. of water; Grassmann, Hannig & Ploéckl, 1955), blotted and laid between polythene sheets so as to leave exposed only the area where the spots were to be put on. This prevented the rest of the paper from drying while the samples (5 μl.) were applied to the starting line 5 cm. from the cathode end. Electrophoresis was carried out at 8 v/cm. for 8 hr. to separate glutamic acid and oxothialmic acid, and for 3.5 hr. when it was necessary to prevent 5-oxopyrrolidine-2-carboxylic acid from migrating off the paper. The dried paper was dipped (six times) through ninhydrin (0.4%, w/v) and cobalt chloride (0.2%, w/v) in propan-2-ol (Wiggins & Williams, 1952) and excess of reagent allowed to drain off; the paper was heated at 90° for 5 min., held in a jet of steam for 3 min. (exposing the whole surface on both sides to the steam), and then left in the dark for 48 hr. The intensity of the spots was measured as previously described (Waley, 1957). Standard solutions have to be run on each sheet; a calibration curve is shown in Fig. 1; 5 μl. of a mm-solution (5 μm-moles) can be estimated with an error of about 5%.

RESULTS

Units of rate

The unit of substrate or product concentration is μM, of enzyme concentration grams (wet weight) of tissue/ml., and the unit of time is the minute; all rates given are expressed in these units, which for brevity are omitted below.

Synthesis of oxothialmic acid from its constituent amino acids

The system was similar to that used by Johnson & Bloch (1951) for the synthesis of glutathione; they found it necessary to reconvert the ADP formed during peptide-bond synthesis into ATP, as ADP inhibited the synthesis of glutathione, and so they added 3-phosphoglyceric acid and a muscle preparation. Even dilute lens extracts catalyse the phosphorylation of ADP by 3-phosphoglyceric acid, so that the muscle preparation can be omitted in the synthesis of glutathione or oxothialmic acid by lens extracts.

The product of the reaction was identified as oxothialmic acid by its chromatographic and electrophoretic mobility. This was confirmed by isolation. The product was eluted from the electrophoresis paper and further purified by preparative paper chromatography in 72% phenol-3% NH₄ soln. (Waley, 1957). Hydrolysise, followed by quantitative paper chromatography, gave the molar ratios of the amino acids in the hydrolysate as glycine 1:0, z-amino-n-butyric acid 1:1, glutamic acid 1:0. Moreover, comparison of the X-ray powder photograph of the copper salt with that of an authentic specimen (for which we are indebted to Mrs D. M. Hodgkin, F.R.S., and Dr B. M. Oughton) confirmed that the product was oxothialmic acid.

The rates of synthesis of oxothialmic acid in several experiments are given in Table 1; the rates did not change appreciably during the reactions, at least during the early stages. The rate varied somewhat with different batches of lenses, and so the same batch was used for any important comparison, as is shown in Table 1. The rate of synthesis of oxothialmic acid was the same whether DL-z-amino-n-butyric acid (16 mm) or L-z-amino-n-butyric acid (8 mm) was used; hence probably only

![Fig. 1. Calibration curve for the reaction of oxothialmic acid with ninhydrin, measured on paper.](image-url)
the L-form is utilized, and the D-form does not compete under these conditions. The rate of synthesis was about the same whether ‘whole lens’ or ‘dialysed lens’ preparations from either the calf or the rabbit were used.

Interaction between the synthesis of ophthalmic acid and of glutathione. Lens batches A and B were used to investigate competition when both ophthalmic acid and glutathione are being synthesized simultaneously. When the concentration of L-cysteine (freshly prepared by neutralization of the hydrochloride) was 8 mM the rate of synthesis of ophthalmic acid was appreciably reduced (Fig. 2; Table 1); in this experiment the GSH synthesized was oxidized as described to GSSG before electrophoresis.

Formation of 5-oxopyrrolidine-2-carboxylic acid. 5-Oxopyrrolidine-2-carboxylic acid is also formed in the course of the synthesis of ophthalmic acid at a rate of about 3 units. This rate was determined only by assessment of the intensity of the spots obtained by paper chromatography in butanol-acetic acid-water (40:9:20, by vol.), followed by treatment of the chromatogram with chlorine and o-tolidine (Reindel & Hoppe, 1954); the error is probably about 50%. 5-Oxopyrrolidine-2-carboxylic acid was identified by comparison with a sample prepared from glutamic acid (Ellfolk & Synge, 1955), and by its hydrolysis to glutamic acid.

Breakdown of γ-glutamyl peptides

A solution (2 ml.) containing dialysed calf lens (1-54 ml.) and γ-L-glutamyl-L-α-amino-n-butyric acid (17 μmoles) was kept at 37° and pH 6-4. Paper electrophoresis showed the disappearance of the starting material and the appearance of only one ninhydrin-positive product, α-amino-n-butyric acid; no glutamic acid was formed. When the paper was treated with the chlorine-o-tolidine reagent (Reindel & Hoppe, 1954) the other product of the reaction was seen to be 5-oxopyrrolidine-2-carboxylic acid. The reaction is

\[
\begin{align*}
\text{HN} & \cdot \text{CH(CO}_2\text{H}) & \cdot \text{[CH}_2\text{]}_2 \cdot \text{CO} \cdot \text{NH} & \cdot \text{CH}_2\text{Et} & \cdot \text{CO}_2\text{H} \\
\to \\
\text{HN} & \cdot \text{CH(CO}_2\text{H}) & \cdot \text{[CH}_2\text{]}_2 \cdot \text{CO} & \cdot \text{H}_2\text{N} & \cdot \text{CH}_2\text{Et} & \cdot \text{CO}_2\text{H}
\end{align*}
\]

The rate of breakdown of the dipeptide was 182 units; when the lens preparation was diluted 10 times the rate of breakdown was 18 units, so that the rate of the non-enzymic breakdown is negligible under these conditions.

A similar reaction occurred with γ-L-glutamylglycine; the rate of breakdown was 30 units.

Dipeptidase activity of lens extracts

Calf lens extracts catalysed the hydrolysis of L-α-amino-n-butyrylglycine; the rate of the reaction was not measured, but it took place more rapidly than any of the other reactions cited (cf. Kinoshita & Masurat, 1957). The dipeptidase of calf lens can be purified by the method which Binkley (1952, 1954) applied to kidney extracts; the unusual properties of this enzyme led Binkley to suggest that it was a polynucleotide, but subsequent work renders this unlikely (Semenza, 1957).

Synthesis of γ-glutamyl-α-amino-n-butyric acid

The substrates were L-glutamic acid (8 mM) and DL-α-amino-n-butyric acid (16 mM); the other constituents of the mixture were ATP (2-5 mM), 3-phosphoglyceric acid (22 mM), KCl (42 mM), MgSO₄ (10 mM), potassium phosphate buffer

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Table 1. Rates of synthesis of ophthalmic acid

<table>
<thead>
<tr>
<th>Lens batch</th>
<th>Animal</th>
<th>Standard conditions</th>
<th>Cysteine added</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Calf</td>
<td>8.7 (±0.8)</td>
<td>4.7 (±0.4)</td>
</tr>
<tr>
<td>B</td>
<td>Calf</td>
<td>10.3 (±1.2)</td>
<td>3.1 (±0.5)</td>
</tr>
<tr>
<td>C</td>
<td>Calf</td>
<td>14.3</td>
<td>10.9</td>
</tr>
<tr>
<td>D</td>
<td>Rabbit</td>
<td>13.8</td>
<td>13.8</td>
</tr>
</tbody>
</table>

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Fig. 2. Formation of ophthalmic acid in the presence (●) and absence (○) of cysteine, followed by paper electrophoresis.
(pH 6.4) (6 mm). The solution (10 ml.) containing dialysed calf lens (7.7 ml.) was kept at 37° and the pH was 6.3. The rate of decrease of the concentration of glutamic acid was 18 units, and the rate of increase of 5-oxopyrrolidine-2-carboxylic acid was about the same. The stationary state concentration of the dipeptide, attained after about 2 hr., was about 0.2 mm.

**Synthesis of ophthalmic acid from \( \gamma \)-glutamyl-\( \alpha \)-amino-\( \eta \)-butyric acid and glycine**

The substrates were \( \gamma \)-L-glutamyl-\( \alpha \)-amino-\( \eta \)-butyric acid (8 mm) and glycine (8 mm); the nature and concentration of the other constituents were the same as in the synthesis of ophthalmic acid from its component amino acids; dialysed calf lens was used. The rate of breakdown of the dipeptide was 180 units, and the rate of synthesis of ophthalmic acid was 63 units (Fig. 3); the formation of 5-oxopyrrolidine-2-carboxylic acid accounts for the difference between the two rates.

**Synthesis of \( \gamma \)-glutamylalaninylglycine and \( \gamma \)-glutamylthreoninylglycine**

Dialysed calf lens was used for these experiments and the incubation mixture resembled that used for the synthesis of ophthalmic acid from its component amino acids, except that \( \alpha \)-amino-\( \eta \)-butyric acid was replaced by one of the following amino acids (8 or 16 mm, depending on whether the \( L \)- or the \( DL \)-form was used): alanine, threonine, valine, serine, homocysteine, homoserine, tryptophan, tyrosine, \( \alpha \)-aminoisobutyric acid, phenylalanine, methionine, \( \beta \)-alanine, isoleucine, leucine, lysine, proline, hydroxyproline, \( \beta \)-aminoisobutyric acid, aspartic acid, glutamine, cystine, asparagine, citrulline, histidine, ornithine, arginine, \( \gamma \)-aminoisobutyric acid, and the cysteine-\( \eta \)-ethylmalimide adduct. Only the first two amino acids gave ninhydrin-positive products of the expected electrophoretic mobility. The products were isolated by elution from the electrophoresis papers, and the amino acid composition was determined by hydrolysis and quantitative paper chromatography. The product from alanine had the same \( R_f \) as norophthalmic acid (Waley, 1957), but it was contaminated with ophthalmic acid (not completely removed by dialysis and so present in the lens preparation) and the amino acids present in the hydrolysate, alanine, glutamic acid, glycine and \( \alpha \)-amino-\( \eta \)-butyric acid were in the ratio 1:0.15:1.6:0.5. The product from threonine gave on hydrolysis threonine, glutamic acid and glycine, in the ratio 0.8:1:0:1.0. We conclude that both products are analogous to ophthalmic acid and glutathione. The rate of synthesis of \( \gamma \)-glutamylalaninylglycine was about 1 unit, and that of \( \gamma \)-glutamylthreoninylglycine about 0.5 unit; these are only approximate values, assessed by inspection of the spots.

**Breakdown of ophthalmic acid in the liver**

Fresh calf liver (50 g.) was macerated with 0.1 m-potassium phosphate (pH 7.4) (69 ml.), 0.16 m-KCl (28 ml.) and 0.16 m-MgSO\(_4\) (3 ml.) at 4°, and a portion of the supernatant (4.0 ml.) used in a volume of 4.1 ml., containing 16 \( \mu \)moles of the potassium salt of ophthalmic acid. The mixture was kept at 37°, at pH 7.3. The usual method of precipitating the proteins with ethanol was not adequate and the samples were subsequently acidified with 1% acetic acid to pH 5.2 and heated at 100° for 3 min. to complete removal of the proteins. Paper electrophoresis was used to measure the rate of breakdown of ophthalmic acid (42 units) and the rates of formation of glutamic acid (40 units) and neutral amino acids (92 units). As complete hydrolysis of ophthalmic acid liberates 2 mol. of neutral amino acids to 1 mol. of glutamic acid, the measured rates suggest a two-step mechanism, in which the second step is faster than the first:

\[
\text{Ophthalmic acid} \rightarrow \text{glutamic acid} + \alpha \text{-amino-\( \eta \)-butyrylglycine}
\]

\[
\alpha \text{-Amino-\( \eta \)-butyrylglycine} \rightarrow \alpha \text{-amino-\( \eta \)-butyric acid} + \text{glycine}
\]
Estimation of ophthalmic acid in tissues

The ophthalmic acid content of tissues was investigated by a two-dimensional procedure: paper electrophoresis was followed by paper chromatography; this ensured a good separation of glutathione (which was oxidized to GSSG during the paper chromatography) and a better characterization of ophthalmic acid. Lenses were ground under ethanol (to give a final ethanol concentration of 70 %, v/v), the protein precipitate spun down, the supernatant evaporated in vacuo and the residue dissolved in aq. 10 % (v/v) propan-2-ol. A portion (5 μl) was used for electrophoresis on a 25 cm. × 44 cm. sheet of Whatman no. 52 paper. Portions (5 μl) of three standard solutions of ophthalmic acid were applied along the starting line for the chromatographic run in positions such that the ophthalmic acid was finally separated from any ninhydrin-positive compounds in the lens extract. Electrophoresis was carried out for 2 hr. (see Methods for further details). The dried paper was run in the second dimension for 24 hr., 72 % (w/v) phenol–3 % (w/v) NH₃ soln. being used as solvent. Glutathione was estimated (as GSSG) after paper chromatography in phenol–NH₃. The results, given in Table 2, are for calf and cow lenses; ophthalmic acid was not detected in rabbit or pigeon lenses, i.e. its concentration is less than 7 μmoles/100 g. of lens, and was possibly present at about this concentration in human lenses. Preliminary experiments failed to reveal ophthalmic acid in other tissues of the calf, but it is hoped to investigate these more thoroughly.

Reaction of pyruvic acid with amino compounds

Ninhydrin-positive substances with high electrophoretic mobility (migrating towards the anode on paper electrophoresis at pH 4) were frequently encountered during the synthesis of the peptide bond, when 3-phosphoglyceric acid is breaking down to pyruvic acid. Such acidic substances were then found to be formed quite rapidly from amino acids or peptides and pyruvic acid in neutral solution at room temperature. It has not been possible to examine these compounds in detail, as the extent of their formation varied unpredictably and they decompose on elution from dried electrophoresis papers, but it seems possible that these substances are the anils known to be formed from pyruvic acid and amines at pH 9 (Zuman, 1951).

DISCUSSION

Synthesis of ophthalmic acid: the overall reaction

Ophthalmic acid and glutathione are structurally similar and both synthesized by lens extracts. These observations suggest that the same enzyme may be responsible for the synthesis of both tri-peptides. This possibility can be tested in the following way. If one enzyme (E) catalyses two reactions, as in the scheme shown below, then it can readily be shown that the rate of formation of the product (B) will be less when both the substrates A and C are present than when only one (A) is, unless the Michaelis constant of A is much less than that of C. The substrates may be pictured

A + E ⇌ AE → B + E
C + E ⇌ CE → D + E

as competing for the same active site on the enzyme, and so the greater the concentration of the enzyme–substrate compound CE the less the concentration of the compound AE, and hence the less the rate of formation of the product B. It was, in fact, found that the rate of synthesis of ophthalmic acid was depressed by the presence of cysteine (Fig. 2). There is, then, competition at one or both stages of the synthesis: cysteine and α-amino-β-carboxylic acid may compete in the first stage, or γ-glutamylcysteine and γ-glutamyl-α-amino-β-carboxylic acid may compete in the second. Thus at least one of the two enzymes involved in glutathione synthesis takes part in the synthesis of ophthalmic acid.

γ-Glutamyl lactamase in lens

Heating γ-linked peptides of glutamic acid in aqueous solution causes cyclization to 5-oxopyrroolidine-2-carboxylic acid (Le Quesne & Young, 1952) and the same reaction is catalysed by an enzyme (γ-glutamyl lactamase) in liver extracts (Hanes & Connell, 1956). Lens extracts were found to effect the conversion of γ-glutamyl-α-amino-β- carboxylic acid (γ-GluAB) into 5-oxopyrroolidine-2-carboxylic acid (PCA) and α-amino-β-carboxylic acid (AB):

γ-GluAB → PCA + AB.  

Table 2. Concentration of ophthalmic acid in lens

<table>
<thead>
<tr>
<th>Lens</th>
<th>Wt. of lens used (g.)</th>
<th>Ophthalmic acid (μmoles/100 g. of lens)</th>
<th>Glutathione (μmoles/100 g. of lens)</th>
<th>Ratio of concn. of glutathione to concn. of ophthalmic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>10.9</td>
<td>118</td>
<td>1220</td>
<td>10.3</td>
</tr>
<tr>
<td>Calf</td>
<td>10.0</td>
<td>121</td>
<td>1060</td>
<td>8.8</td>
</tr>
<tr>
<td>Calf</td>
<td>7.8</td>
<td>56</td>
<td>820</td>
<td>9.5</td>
</tr>
</tbody>
</table>
This reaction is apparently confined to the dipeptide; glutamic acid and ophthalmic acid are unaffected on incubation with lens extracts. The relatively rapid decomposition of \( \gamma \)-glutamyl-\( \alpha \)-amino-\( n \)-butyric acid necessarily affects the overall rate of ophthalmic acid synthesis in crude lens extracts.

The two stages of the synthesis of ophthalmic acid

The view that the first stage of the synthesis of ophthalmic acid is the formation of \( \gamma \)-glutamyl-\( \alpha \)-amino-\( n \)-butyric acid is confirmed by the observation that this dipeptide is indeed formed from the amino acids (and ATP) by lens extracts:

\[
\text{Glu} + \text{AB} \rightarrow \gamma \text{-GluAB}. \quad (2)
\]

Only a low concentration (0-2 mM) of the dipeptide was built up, as it breaks down rapidly by the reaction (1) shown above. The net effect of reactions (1) and (2) is the conversion of glutamic acid into 5-oxopyrrolidino-2-carboxylic acid.

The second stage of synthesis of ophthalmic acid is the reaction of \( \gamma \)-glutamyl-\( \alpha \)-amino-\( n \)-butyric acid with glycine (reaction 3):

\[
\gamma \text{-GluAB} + \text{Gly} \rightarrow \gamma \text{-GluABGly}. \quad (3)
\]

The presence of \( \gamma \)-glutamyl lactamae in lens extracts results in the dipeptide decomposing more rapidly than ophthalmic acid is formed (Fig. 3). The rate of synthesis of ophthalmic acid in this experiment was about five times as great as that from the three amino acids, as the initial concentration of the dipeptide in the first case was much higher than the stationary-state concentration reached in the second.

These results show that the synthesis of ophthalmic acid in lens extracts proceeds by the same two stages as that of glutathione in liver extracts. The formation of 5-oxopyrrolidino-2-carboxylic acid limits the synthesis of ophthalmic acid; it is not known to what extent this also applies to the synthesis of glutathione.

Synthesis and occurrence of other tripeptides

Glutathione and ophthalmic acid are not the only tripeptides occurring in calf lens; norophthalmic acid (\( \gamma \)-glutamylalanylglycine) has been detected (Waley, 1957) and, as hydrolysis of an acidic fraction liberates threonine (Waley, 1956), \( \gamma \)-glutamylthreonylglycine may also be present. The question then arises whether lens extracts will catalyze the synthesis of several peptides of the type \( \gamma \)-Glu(A).Gly, where A is the residue of an amino acid other than cysteine or \( \alpha \)-amino-\( n \)-butyric acid. Out of a wide range of amino acids tested, only alanine and threonine gave rise to tripeptides; the rate of formation of both these peptides was considerably lower than that of ophthalmic acid. Had any of the other amino acids reacted still more slowly, the resulting tripeptide would have been difficult to detect; in fact, there was some evidence that traces of the tripeptide containing valine were formed, but the rate of synthesis was too low to be sure about this. Similarly, on some occasions hydrolysis of the acid fractions of lens extracts released traces of valine (Waley, 1956). There seems, then, to be a parallel between the ability of calf lens extracts to synthesize tripeptides of this type and the occurrence of these tripeptides in the lens. This suggests that the steady-state concentration of these compounds in \( \text{vivo} \) may be governed mainly by their rate of synthesis. Some confirmation of this (although in pathological conditions) is provided by the observation that the decrease in the concentration of glutathione in cataract in rabbit lens is directly proportional to the decrease in the rate of synthesis of the peptide by extracts of these lenses (Daisley, 1955). No breakdown was detectable when either glutathione or ophthalmic acid was incubated with calf lens or rabbit lens extracts, i.e. no catabolic reactions are demonstrable.

The simple idea that the concentrations of these peptides in \( \text{vivo} \) are governed by their rate of synthesis, and that these can be gauged by experiments with tissue extracts, seems to hold well enough for calf lens, but unfortunately lacks generality. No ophthalmic acid could be detected in rabbit lens, and yet the rate of synthesis in rabbit lens extracts is no less than that in calf lens extracts (Table 1). It is, of course, possible that the concentration of \( \alpha \)-amino-\( n \)-butyric acid may be much greater in calf lens than in rabbit lens, but there is no evidence to settle this point.

Metabolism of ophthalmic acid in the liver

A natural extension of the work described above on lens was an investigation whether ophthalmic acid was present, built up or broken down, in another tissue. Liver was chosen, as it contains much glutathione, and liver extracts synthesize glutathione (Snoke & Bloch, 1954). Ophthalmic acid was not detected in calf liver, nor was its synthesis by liver extracts demonstrable, but the rate of breakdown of ophthalmic acid in the extracts was quite high—about four times that of the synthesis by lens extracts. So this may well account for the failure to show net synthesis by liver extracts. Moreover, the breakdown was zero-order, and if it is still zero-order when ophthalmic acid is present at a concentration of 10 \( \mu \)moles/100 g. of tissue, the half-life for the decomposition of ophthalmic acid in the extracts would only be about 1 min., and thus its presence in the liver would not be detected. The decomposition of ophthalmic acid in the liver extracts gave glutamic acid; little
or no 5-oxopyrrolidine-2-carboxylic acid was formed. The direct hydrolysis of the $\gamma$-glutamyl peptide bond has also been observed by Woodward & Reinhart (1942), who found that glutathione was hydrolysed to glutamic acid by kidney extracts in the pH range 5–7; in more alkaline solution, however, formation of 5-oxopyrrolidine-2-carboxylic acid was observed. Cyclization to the oxopyrrolidine occurs preferentially in alkaline solution because only the uncharged amino group is nucleophilic; formation of intermolecular amide, too, is favoured (at the expense of hydrolysis) by raising the pH (Fruton, 1955–56).

**SUMMARY**

1. Lens extracts catalyse the synthesis of the tripeptide, ophthalmic acid, from its constituent amino acids and adenosine triphosphate. The synthesis proceeds through the intermediate formation of $\gamma$-glutamyl-$\alpha$-amino-n-butyric acid.

2. Either (or both) of the enzymes of glutathione synthesis are also involved in the synthesis of ophthalmic acid.

3. Analogous tripeptides are synthesized when $\alpha$-amino-n-butyric acid is replaced by alanine or threonine.

4. Liver extracts catalyse the hydrolysis of ophthalmic acid to its three amino acids.

5. Rabbit lens extracts contain little, if any, ophthalmic acid, but catalyse its synthesis just as effectively as do calf lens extracts.

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**REFERENCES**


