A New Technique for the Estimation and Isolation of the Hexone Bases in Protein Hydrolysates

By W. ROBSON AND A. S. M. SELIM

Department of Physiology and Biochemistry, King's College, Strand, London, W.C. 2

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The difficulties inherent in the method of Kossel & Kutscher (1900) for isolating the hexone bases from gelatin hydrolysates as phosphotungstates may be partially overcome by the direct precipitation of arginine and lysine from such mixtures as their picrates (Robson & Selim, 1952). Hydroxylysine and histidine cannot, however, be likewise precipitated from gelatin hydrolysates. To overcome this difficulty we have elaborated the present method which may be used not only for the purpose of isolating the individual hexone bases but also for estimating them in small amounts of protein.

Of the many new methods introduced in recent years for isolating amino-acids from protein hydrolysates, those based on adsorption have received much attention. Active carbon preparations capable of adsorbing amino-acids from solutions have long been known, but the main application of the use of this reagent has been the quantitative removal of the aromatic amino-acids from protein hydrolysates (Schramm & Primosigh, 1943; Tiselius, Drake & Hagdahl, 1947; and Partridge, 1949). Warburg & Negelein (1921) and others have shown that certain types of charcoal oxidize amino-acids and that such oxidation may be prevented or reduced by treatment of the charcoal with hydrogen cyanide, sodium cyanide or hydrogen sulphide. From the data collected during the present investigation it would appear that such treatment of the charcoal does not affect the results to any appreciable extent.

It is well known that picric acid is strongly adsorbed by charcoal. We have now found that arginine monopicrate and histidine dipicrate are even more strongly adsorbed than picric acid itself, 5 g. of charcoal (treated as described in the experimental section) being capable of adsorbing 1 g. of the former salt. On the other hand, the picrates of lysine, hydroxylysine and ammonia, in comparison, are not so strongly adsorbed. Certain neutral amino-acids, e.g. glycine, proline, etc., form picrates under specific conditions. Passage of such picrates through a charcoal column results in the cleavage of the salt link, the picric acid being retained by the charcoal, the amino-acid moving down and out of the column if sufficient eluate is employed. This can be shown by applying a solution of glycine picrate to

a column of charcoal. On washing the column with sufficient water, all of the glycine will ultimately appear in the eluate which remains colourless, indicating that the picric acid has been retained by the charcoal.

These facts form the essential basis of the present method. It consists briefly of two stages: first, the removal of the aromatic amino-acids by the passage of the protein hydrolysate, pH 2-3, over a column of charcoal followed by treatment of the column with water until the eluate gives a negative ninhydrin reaction; secondly, the conversion of the bases of the combined filtrate and eluate, pH 7-8, into their picrates by the solution therein of several moles of picric acid (Table 1). The mixture is next passed through a column of charcoal which is then washed with water until the eluate gives a negative ninhydrin reaction. By the latter process, the picrates of the hexone bases are retained on the charcoal while the free monoamino-acids and the acidic amino-acids pass out in the eluate.

Treatment of the column with 0.5N-hydrochloric acid or sulphuric acid decomposes the adsorbed picrates, the bases appearing in the percolate and the picric acid being retained by the charcoal. In this process the less well-adsorbed hydroxylysine, lysine and ammonia appear first in the percolate and a gap, varying in volume according to the length of the column, occurs, as shown by the negative ninhydrin reaction before the more strongly adsorbed histidine and arginine are eluted. The two former bases can thus be separated from the latter and estimated by the micro-Kjeldahl and periodate techniques.

When the hydroxylysine and lysine have been removed from the column, the eluant is changed to N-hydrochloric acid or N-sulphuric acid. The resulting percolate first contains histidine, then a mixture of histidine and arginine and finally arginine. The last traces of arginine on the lowest quarter of the column are removed by boiling it with dilute acid. Histidine and arginine in the combined percolate and extracts are estimated colorimetrically.

For the purpose of isolating the individual bases in quantity the above technique is slightly varied. As is proved in the experimental section, histidine is adsorbed only as its dipicrate. To the hot protein

hydrolysate, freed from aromatic amino-acids as described above, picric acid equivalent to 1 mole for the arginine and 2 moles for the histidine present (an approximate estimation of these amino-acids having been made colorimetrically) is added. When the picric acid has dissolved the mixture is cooled and passed through a column of charcoal. The column is washed with water until all the mono-amino-acids, together with the lysine, hydroxylysine and ammonia, have passed out. The column is now treated with 0.01–0.1N-ammonia which decomposes histidine dipicrate. The removal of the histidine from the column is followed colorimetrically. When the last stage is complete, the column is washed with N-hydrochloric acid until the eluate gives only a faint Sakaguchi reaction. At this point the lowest quarter of the column is boiled with more of the eluant to remove the last traces of arginine. The histidine and arginine, separated in this way, were identified as the diflavinate and monoflavinate respectively.

Compared with the picrates of arginine and histidine, those of lysine and hydroxylysine are only weakly adsorbed on charcoal. Several molar quantities of picric acid in excess of those theoretically required for the formation of the picrates of lysine and hydroxylysine are dissolved in the mixture now free from aromatic amino-acids, histidine and arginine. The mixture is passed through a charcoal column as before, and the column washed with water until the eluate no longer gives a positive ninhydrin reaction. The column is now treated with 0.5N-sulphuric acid until the eluate gives a negative ninhydrin reaction. The combined eluate and washings, freed from sulphuric acid (pH 10), is concentrated and picric acid dissolved in the hot solution until the latter has a pH value of 4.5. The crystalline picrate which separated melted at 220–230° and was a mixture of the picrates of lysine and hydroxylysine. One recrystallization raised the temperature of decomposition to 261°, identical with that of lysine picrate. On cooling the concentrated mother liquor, a bright yellow crystalline picrate was obtained in excellent yield, m.p. 226–227°. Analysis confirmed that it was the picrate of hydroxylysine.

The removal of the phosphates and calcium from the charcoal before use should be thorough. Any phosphoric acid remaining will tend to decompose the picrates of the basic amino-acids. Calcium, as will be shown in a later paper, forms insoluble complex salts with monoamino-acid picrates, and these may be retained on the charcoal. Further investigation has shown that the method is generally applicable to other proteins besides gelatin and, for purposes of estimation, to much smaller quantities than those mentioned below. Further results will be reported later.

EXPERIMENTAL

Materials

Gelatin (Coignet's Gold Label Leaf) was used throughout the investigation. (N, 17.17 g./100 g. of moisture-free protein; moisture, 15.4 %.)

Picric acid. Commercial picric acid was purified by the method of Benedict (1929).

Charcoal. The charcoal used in all experiments was a British Drug Houses active decolorizing charcoal. It was purified before use by the following procedure. The charcoal was boiled for 1 hr. three times with 3N-HCl, three times with distilled water and dried in the oven at 150°. It was further boiled twice with water and finally dried at 150° for 48 hr. An aqueous extract of the final product should have a pH not less than 5 and should give negative tests for both phosphates and calcium.

Methods of analysis

All N determinations were carried out by the micro-Kjeldahl procedure on samples of suitably diluted solutions containing between 0.2 and 0.5 mg. of N. Digestion lasted 20 hr.

Arginine was determined colorimetrically by the Brand & Kassell (1942) modification of the Sakaguchi reaction. All colorimetric estimations were carried out on the EEL portable colorimeter using green filter 624.

Histidine was determined colorimetrically by Macpherson's modification (1942, 1946) of the Pauly reaction.

Hydroxylysine was determined by estimating the NH_3 produced upon treatment of a sample of the unknown solution with alkaline periodate. The procedure employed followed closely that of Van Slyke, Hiller & MacFadyen (1941), except that the NH_3 produced from the reaction was first aerated into excess H_2SO_4 and then estimated by micro-Kjeldahl distillation.

Lysine was estimated by determining the difference between the N of the lysine-hydroxylysine fraction and the hydroxylysine N as determined above.

Proof that histidine is only adsorbed in the form of its dipicrate

On applying a neutral gelatin hydrolysate, free from its basic amino-acids, and in which histidine hydrochloride (100 mg.) and picric acid (1 equiv.) had been dissolved, to a column of active charcoal (10 g.) the percolate was found to be free from histidine. When a 5% aqueous acetic acid solution, which unlike mineral acids does not decompose histidine dipicrate, was allowed to pass through the column the eluate contained histidine. Development of the column with acetic acid was continued until no more histidine was eluted. At this stage the eluate was changed to N- H_2SO_4 . Development of the column with a sufficient volume of this solution gave a percolate containing the remainder of the histidine. This experiment showed that the histidine had been adsorbed partly as such and partly as dipicrate (cf. Ewins & Pyman, 1911).

The above experiment was repeated, but the amount of picric acid added was increased to 2 equiv. On treating the column with aqueous acetic acid the effluent did not contain any histidine. Treatment, however, with N- H_2SO_4 removed the histidine completely.

*Estimation of the basic amino-acids
in gelatin hydrolysates*

Gelatin (25 g.) was hydrolysed by boiling it with 160 ml. of $8N-H_2SO_4$ for 30 hr. The insoluble humin was filtered off and the filtrate diluted to about 0.5 l. with water, and neutralized to pH 2.5 with an aqueous suspension of $Ca(OH)_2$. The $CaSO_4$ precipitate was removed and extracted twice with boiling water. The filtrate and washings were combined (2 l.) and passed through a column of active charcoal (10 g. in a glass tube of 2.5 cm. internal diam.). The colourless percolate was free from humin and from tyrosine and phenylalanine, as indicated by the negative tests with the Bernhart (1938) modified Millon-Weiss reaction and by the Kuhn & Desnuelle (1937) modified Kapeller-Adler reaction. The column was washed with 1 l. of $0.2N-H_2SO_4$ and the acid effluent added to the main percolate. Most of the H_2SO_4 was removed from the combined liquors with $Ca(OH)_2$ and the remaining SO_4^{2-} removed with $Ba(OH)_2$. Any Ca ions remaining in solution were removed with oxalic acid, the precipitate each time being extracted twice with boiling water. To the combined filtrate and washings (approx. 4.5 l.) picric acid (20 g.) was added, brought into solution by warming, and the mixture allowed to cool to room temperature. It was then applied to a column of charcoal (100 g., 4×50 cm.) at a rate of about 1 l./3 hr. The column was washed with water, until the percolate gave a negative ninhydrin reaction. Volume of eluate was about 10 l. The column was then treated with $0.5N-H_2SO_4$ and the percolate tested with ninhydrin. The first 1.5 l. of the acid percolate gave a strong ninhydrin reaction. The following 0.5–1.0 l. gave a weak test, and finally a negative test was obtained for another 0.5 l. The percolate contained lysine and hydroxylysine. Further development with the same eluant gave an eluate which contained histidine (positive ninhydrin and modified Pauly tests). At this point the eluant was changed to $N-H_2SO_4$. The first fractions of stronger acid contained only histidine, but later fractions contained histidine and arginine. Treatment of the column with $N-H_2SO_4$ was continued until the test for arginine was very weakly positive. About 7 l. of acid solution containing histidine and arginine were collected. The column of adsorbate was then extruded and its lowest quarter separated and extracted twice with boiling $N-H_2SO_4$ to remove the last trace of arginine. The filtrate from this operation was added to the arginine-histidine eluate, made up to volume, and determinations of these two bases carried out colorimetrically.

Estimation of lysine and hydroxylysine. The fraction containing lysine and hydroxylysine was neutralized with $Ca(OH)_2$, the $CaSO_4$ precipitate collected, extracted with boiling water and the filtrate and extract combined. A further quantity of $Ca(OH)_2$ was added to the solution to make it strongly alkaline and the mixture concentrated *in vacuo* at a low temperature, the NH_3 being removed in the process. The solution was finally made up to a volume of 1 l., and samples removed for determinations (Kjeldahl) of total N.

The alkaline solution was then further concentrated *in vacuo* at a low temperature and made up to a volume of 250 ml. Samples were taken for the estimation of the periodate NH_3 .

The results of several experiments using different quantities of picric acid are shown in Table 1.

Method of isolation of the bases

Since arginine and histidine are much more strongly adsorbed on charcoal than lysine and hydroxylysine it follows that when picric acid (1 mole/mole of arginine plus 2 moles/mole of histidine) is dissolved in a protein hydrolysate, and the mixture is applied to a charcoal column, arginine and histidine will be quantitatively retained on charcoal as their picrates, while free lysine and hydroxylysine will pass unadsorbed.

Gelatin (500 g.) was hydrolysed by boiling it for 30 hr. with 3 l. of $8N-H_2SO_4$.

Removal of the aromatic amino-acids. The hydrolysate was diluted with 2 vol. of distilled water and the humin removed by filtration. The pH of the mixture was raised to 2.5 by addition of a suspension of $Ca(OH)_2$ and the precipitated $CaSO_4$ collected and extracted thoroughly with boiling water. The combined filtrate and extracts (approx. 10 l.) was divided into two equal portions and each passed through a column of charcoal (60 g.; 4 cm. internal diam.). The charcoal had been previously treated with 1% HCN. The columns were washed with $0.2N-H_2SO_4$ until a negative test for arginine was given by the effluent. The eluates from the two columns were combined and the remaining SO_4^{2-} and Ca^{2+} completely removed, by successive precipitation with $Ca(OH)_2$, $Ba(OH)_2$ and oxalic acid. The precipitate in each case was extracted with boiling water. The combined filtrate and extracts had a volume of about 20 l. and a pH of approximately 8.

Removal of arginine and histidine. Estimations showed the presence in the solution of 35.15 g. of arginine and 3.0 g. of

Table 1. *Results of the estimation of the basic amino-acids in gelatin*

(The figures emphasize that the maximum values for lysine and hydroxylysine are only achieved by using more moles of picric acid than are required for the formation of the monopicrates of these bases. The data quoted are N as % of protein N.)

Picric acid (g.)	Charcoal (g.)	Arginine	Histidine	Lysine	Hydroxylysine
6.6	40	15.24	1.05	1.54	—
11.4	65	—	—	3.74	0.46
14.0	75	—	—	4.09	0.75
19.0	90	—	—	4.58	0.84
20.0	100	15.21	1.01	4.51	0.84
20.0	100	15.16	0.97	4.63	0.89
20.0	100*	15.22	1.04	4.66	0.89

* Charcoal pretreated with 1% aqueous HCN.

histidine. Picric acid (56 g., 5% excess) was dissolved in the hydrolysate, and the mixture applied to a column of HCN-pretreated active charcoal (400 g., tube 8 cm. internal diam.) at a rate of about 1.5 l./hr. The resulting colourless percolate was entirely free of arginine and histidine as indicated by negative Sakaguchi and Pauly tests. The column was washed with distilled water until the eluate gave a negative ninhydrin reaction. Volume of water used was 30 l. The picrates of arginine and histidine were ultimately worked up according to the technique described below.

To the combined percolate and washings (50 l.) picric acid (320 g.) was added and the mixture stirred until all the reagent was dissolved. The solution was divided into two equal portions and each applied to a column of HCN-pretreated charcoal (850 g., tube diam. 8 cm.), at the rate of approx. 500 ml./hr. Water was percolated through each column until the eluate gave a negative ninhydrin reaction. Each column was treated with 0.5N- H_2SO_4 to elute lysine and hydroxylysine. Elution with acid was stopped when the effluent gave a negative ninhydrin reaction. About 40 l. of the acid eluate were collected from each column. The combined eluates were freed from H_2SO_4 by the $\text{Ca}(\text{OH})_2$, $\text{Ba}(\text{OH})_2$, oxalic acid technique. The various precipitates were extracted with boiling water, the combined filtrates and washings (pH 10) concentrated *in vacuo* to about 500 ml. and filtered. Solid picric acid (28.5 g.) was added in portions to the hot concentrate and dissolved by stirring until the pH of the mixture had fallen to 4.5–5.0. During the addition, crystals (lysine picrate) began to separate and when the process was complete the mixture, still hot, was semi-solid. After 2 days at 0° the mixture was filtered and the collected picrate washed first with a little ice-cold water and then with ethanol. The product, decomposition point 220–230°, was

recrystallized from water (500 ml.). After 2 days at 0° the product was collected, washed with ice-cold water and ethanol and finally dried at 110°. It weighed 38.0 g. and, on heating, decomposed explosively at 261°, characteristic of lysine picrate. The yield obtained corresponded to 3.5 g. lysine/100 g. moisture-free gelatin.

The filtrates and washings from these two operations were combined, concentrated to a small volume and set aside overnight at 0°. The bright-yellow crystalline picrate which had separated was collected at the pump, washed with a little ice-cold water and finally with absolute ethanol. Dried in the oven at 110° it decomposed on heating at 226–227°. The filtrate, on further concentration, yielded a small crop of similar crystals. Hydroxylysine picrate melts at 226–227° (Martin & Synge, 1941; Sheehan & Bolhofer, 1950). A few mg. of the substance treated with alkaline periodate and dimedone gave a product which, recrystallized from ethanol, melted at 185° (melting point of the dimedone-formaldehyde condensation derivative, 189°). Admixture of the product with an authentic sample of the condensation product gave no lowering of the melting point. The total yield of pure hydroxylysine picrate obtained was 5.8 g., equivalent to 0.57 g. hydroxylysine per 100 g. moisture-free gelatin.

Preparation of hydroxylysine monohydrochloride. Hydroxylysine picrate (1 g.) was decomposed with hot dilute HCl and the liberated picric acid removed by extraction with successive portions of hot benzene. The acid aqueous solution was concentrated to a syrup *in vacuo*, the residue taken up in hot water, clarified with charcoal and again concentrated to a thick syrup which was taken up in hot ethanol. Pyridine was added until a slight excess was present and more hot ethanol added. On scratching the side

Table 2. *The hexone base content of gelatin hydrolysates as estimated by the present method compared with previously reported figures*

(Data quoted are N as % gelatin N.)			
Amino-acid	Value	Method	Reference
Arginine	15.20	Present	—
	15.3	Electrodialysis, colorimetry and alkaline digestion	Macpherson (1946)
	11.6	Electrodialysis, flavianic acid precipitation	Albanese (1940)
	15.3	Diflavanate precipitation	Vickery (1940)
	14.6	Microbiological	Hier, Graham, Freides & Klein (1945)
	15.7	Microbiological	Neuman (1949)
Histidine	1.0	Present	—
	1.1	Electrodialysis and colorimetry	Macpherson (1946)
	0.76	3:4-Dichlorobenzenesulphonic acid precipitation	Vickery & Winternitz (1944)
	0.87	Microbiological	Stokes, Gunness, Dwyer & Caswell (1945)
	1.0	Microbiological	Neuman (1949)
Lysine	1.2	Microbiological	Hier <i>et al.</i> (1945)
	4.6	Present	—
	4.9	Electrodialysis and N difference	Macpherson (1946)
	4.5	Microbiological	Hier <i>et al.</i> (1945)
	5.4	Microbiological	Neuman (1949)
Hydroxylysine	6.2	Microbiological	Stokes <i>et al.</i> (1945)
	0.87	Present	—
	1.2	Electrodialysis-periodate	Rees (1946)
	0.9	Phosphotungstic acid precipitation, periodate estimation	Van Slyke <i>et al.</i> (1941)

Table 3. Amounts of lysine and hydroxylysine isolated from gelatin hydrolysates by present method compared with values previously reported in the literature

(Values quoted are g. amino-acid/100 g. moisture-free gelatin.)

Amino-acid	Value	Method	Reference
Lysine	3.50	Present	—
	2.91	Benzylidene-Cu precipitation	Turba (1948)
	2.72	Benzoyl-Cu precipitation,	Kurtz (1941)
Hydroxylysine	0.57	Present	—
	0.57	Resin exchange-alumina adsorption	Sheehan & Bolhofer (1950)
	0.034	Phosphotungstic acid precipitation, acetylation-benzoylation	Martin & Synge (1941)
	0.004	Electrodialysis, fractional crystallization of the picrate	Heathcote (1948)

of the vessel small rod-like crystals separated. Crystallization was allowed to continue overnight at 0°. The monohydrochloride was collected, washed with absolute ethanol and recrystallized from an aqueous ethanol mixture. The dry product (0.37 g.) had m.p. 229–231°. Further recrystallization affected no change in the melting point. Sheehan & Bolhofer (1950) give 215–220°, but these workers regarded their product as partially racemized. Heathcote (1950) gives 233.5° (corr.). (Found: N, 13.97. Calc. for $C_6H_{14}O_3N_2$: HCl, 14.1 %.)

Isolation of histidine and arginine

The procedure is based on the fact that dilute NH_3 solution decomposes histidine dipicrate but not arginine picrate.

Gelatin (50 g.) was hydrolysed with H_2SO_4 as above. The acid was partially neutralized with an aqueous suspension of $Ca(OH)_2$ until the pH of the mixture was 2.5, and the filtered solution passed over a column of charcoal (20 g.), to remove humin and the aromatic amino-acids. The column was washed with 1.5 l. of 0.2N- H_2SO_4 . The remainder of the SO_4^{2-} and Ca^{2+} still in the hydrolysate were exactly removed by the requisite additions of $Ca(OH)_2$, $Ba(OH)_2$ and oxalic acid successively. To the combined filtrate and washings (5 l.) picric acid (5.6 g.) was added and dissolved by warming. The mixture was cooled and applied to a column of charcoal (55 g. pretreated with 1% HCN, diam. 4 cm.) at the rate of 1 l./hr. The column was developed with water until a negative ninhydrin test was obtained on the effluent solution. It was then treated with 0.1N- NH_3 solution until all the histidine was washed out. The ammonia eluate (light brownish due to elution of coloured materials from the charcoal by NH_3) was concentrated *in vacuo*. The concentrated solution was filtered and the volume made up to about 500 ml. with water. The solution, made just acid to Congo red with H_2SO_4 , was decolorized with 0.1 g. of charcoal. The filtrate and washings from the charcoal were concentrated *in vacuo* to about 20 ml. Flavianic acid (15 g.) was added and dissolved by heating. After standing overnight at room temperature, the yellow crystalline precipitate was collected, washed with absolute ethanol and recrystallized from a little hot aqueous flavianic acid solution. The histidine diflavinate collected, washed with absolute ethanol and dried at 110°, melted at 251° (Vickery, 1927). The column, still containing the arginine picrate, was washed with water to remove the excess of NH_3 and then treated with N-HCl until practically all the arginine had been eluted. Excess HCl

was removed *in vacuo* and the thick syrup taken up in 500 ml. of water and neutralized with NaOH until just acid to Congo red. The hot solution was then treated with flavianic acid (7.5 g.). Arginine monoflavinate immediately separated from the solution as orange crystals with the characteristic golden yellow lustre. After 18 hr. at 0° the flavinate was collected, washed with ice-cold water and absolute ethanol and dried at 110°; yield, 8.3 g. of arginine monoflavinate decomposing at 258–259° (Kossel & Gross, 1924).

Isolation of arginine monohydrochloride. In another experiment (50 g. gelatin), the charcoal column still containing the arginine was treated with N- H_2SO_4 until most of the arginine was eluted. The arginine solution was then completely freed from H_2SO_4 with $Ba(OH)_2$. The filtrate and washings from $BaSO_4$ were concentrated *in vacuo* to 500 ml. The alkaline solution was neutralized to pH 6 with dilute HCl and further concentrated to a thick syrup. Arginine monohydrochloride was precipitated by slow addition of absolute ethanol with stirring. It was recrystallized by redissolving in the minimum volume of water and reprecipitating by addition of absolute ethanol. The recrystallized product weighed 2.8 g. and melted at 216–217° (Cox, 1928).

SUMMARY

1. A new technique for the estimation and isolation of the hexone bases in protein hydrolysates is described. It possesses the advantages that it does not involve the use of any expensive reagent or the need for special equipment.

2. The present method not only makes it possible to isolate and separate the individual basic amino-acids in excellent yield and high purity, but it also provides a fraction containing all the basic amino-acids suitable for the application of any of the accepted methods of the base analysis.

3. The introduction of undesirable inorganic and organic reagents is avoided, and after the removal of the bases the hydrolysate is left in a form suitable for the isolation of monoamino- and dicarboxylic amino-acids.

4. The quantity of lysine isolated in the form of the pure picrate from gelatin hydrolysates by the present method exceeds that previously reported in the literature (Table 3).

5. The method permits the isolation of pure hydroxylysine monopicrate in a yield as high as that reported by Sheehan & Bolhofer (1950), although the procedure applied here is much more simple, and yields a product which is not racemized.

6. The new technique adopted for estimation of the bases is superior to the method of electro-dialysis in that lysine is obtained separately. The estimation of this base will not therefore reflect the errors

cumulating from the colorimetric estimations of arginine and histidine, as does the former method.

7. Tables comparing the results obtained in the present work with those previously reported are given.

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Reduction of Glutathione Coupled with Oxidative Decarboxylation of Malate in Cattle Lens

BY RUTH VAN HEYNINGEN AND ANTOINETTE PIRIE
Nuffield Laboratory of Ophthalmology, University of Oxford

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Substances containing sulphhydryl (SH) groups have frequently been considered to be important for the maintenance of the clarity of the lens (see Bellows, 1944). Lens contains about 400 mg. reduced glutathione (GSH)/100 g. lens and one of the two major soluble lens proteins, β -crystallin, has SH groups in the molecule. Lens proteins show greater organ than species specificity so it seems permissible to generalize, at least for the lenses of mammals. Completely cataractous lens contains neither GSH nor any protein SH. It is this fact which has for long suggested that there is some connexion between lens clarity and SH groups, although neither glutathione nor β -crystallin has been shown to take part in any enzyme process occurring in lens. Nor

has it been shown that loss of SH groups precedes rather than follows lens opacity.

Herrmann & Moses (1945) showed the presence of a system in lens which reduced oxidized glutathione (GSSG). The system was unstable and lost its activity after 3 hr. at room temperature; it could be reactivated by boiled extracts of corneal epithelium, kidney or liver but not by glucose and diphosphopyridine nucleotide (DPN). Mapson & Goddard (1951) and Conn & Vennesland (1951) have shown that GSSG is enzymically reduced by reduced triphosphopyridine nucleotide (TPNH) in plant extracts. Rall & Lehninger (1952) have demonstrated the presence of a similar reductase in animal tissues. Mapson & Goddard showed that