storage-tissue slices provided evidence consistent with the proposition that sucrose phosphate is an intermediate in sugar accumulation.

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Amino Acids and Related Compounds in the Lens

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During the course of the fractionation of extracts of calf lens that led to the isolation of *S*-($\alpha\beta$ -dicarboxyethyl)glutathione (Calam & Waley, 1963) several unidentified compounds were detected. Two of these have now been isolated, by ion-exchange chromatography followed by paper electrophoresis or paper chromatography, and identified; one is probably the *O*-phosphate of hydroxylysine, and the other is the mixed disulphide derived from cysteine and glutathione.

An improved general method for the analysis of the ninhydrin-positive compounds in lens extracts was outlined by Calam (1962). The application of this method, which consists of paper electrophoresis at pH 1.6 in one direction followed by paper chromatography in the second direction, to bovine and human (cataractous) lenses is now described in detail. Specific reagents have revealed the presence of ergothioneine, and of several other compounds not previously known to be present in lens. A quantitative analysis of the more abundant amino acids has also been carried out.

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METHODS

Paper electrophoresis. This was carried out in a ridge-pole apparatus (Cliffe & Waley, 1958), usually at 8v/cm., on Whatman papers. The buffers used were: pyridine acetate, pH 4 (50 ml. of acetic acid, 15 ml. of pyridine and 2.5 l. of water; Grassmann, Hannig & Plöckl, 1955) (no. 52 paper); formic acid-acetic acid-water, pH about 1.6 (125 ml. of 90% w/v, formic acid and 375 ml. of acetic acid in 2.5 l.) (no. 3 paper); 10% (v/v) acetic acid, pH about 2.3; 0.05M-veronal, pH about 8.4 (10.3 g. of sodium diethylbarbiturate and 2.8 g. of diethylbarbituric acid/l.). Continuous electrophoresis was carried out at pH 4 in a Beckman model CP apparatus (Calam & Waley, 1963).

Paper chromatography. The solvents used were: butan-1-ol-acetic acid-water (40:9:20, by vol.); butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.) (Waley & Watson, 1953); 72% (w/v) phenol-aq. 3% (w/v) NH_3 soln. Chromatography on cation-exchange papers (Amberlite SA-2) was carried out with 0.2M-acetate buffer, pH 5.2 (Tuckerman, 1958). Whatman no. 1 or no. 4 papers were usually used; for the two-dimensional 'maps', no. 3 paper was used. Amino compounds were detected with ninhydrin (0.4%) and CoCl_2 (0.2%) in propan-2-ol (Wiggins & Williams, 1952); this reagent gives red colours which do not fade. The Sakaguchi, Pauly and molybdate reagents were as described by Smith (1960), the Ehrlich reagent as de-

scribed by Dalglish (1955), and the periodate as described by Metzenberg & Mitchell (1954). The two-dimensional maps were examined in ultraviolet light (254 m μ) before dipping.

Acid hydrolysis. This was carried out with 6N-HCl in sealed tubes for 16 hr. at 110°.

RESULTS

Fractionation of lens extract by ion-exchange chromatography

Calf lenses (217 g., stored frozen for up to 1 year) were macerated under cooled 7% (w/v) trichloroacetic acid (700 ml.) and the supernatant was freed from trichloroacetic acid (Calam & Waley, 1963), concentrated to 60 ml. and brought to pH 7.6 with N-sodium hydroxide. The solution was applied to a column (30 cm. long $\times$ 2.25 cm. diam.) of Dowex 1 (X4; acetate form; 200–400 mesh); the column was washed with 300 ml. of water, and then eluted with pyridine formate (4.2 ml. of 90%, w/v, formic acid and 16.2 ml. of pyridine to 1 l.; 0.1 M with respect to formic acid). Fractions (about 11 ml.) were collected every 15 min. and amino compounds determined by the ninhydrin reaction (Waley & van Heyningen, 1962). There was a small peak (D) spread over fractions 7–14 (about 15 μ moles of amino compounds); the bulk of the acidic compounds (mainly glutamic acid and ophthalmic acid) were collected in fractions 20–44 (E) (see below).

Constituents of fraction 'D'. The contents of fractions 7–14 were concentrated to a volume 0.5 ml. and applied as a thin band (25 cm. long) to no. 3 paper previously moistened with formic acid-acetic acid buffer. Electrophoresis was then carried out for 190 min. at 300 v. The ninhydrin-positive fractions (Fig. 1) (located by a guide strip) were eluted with water by the method of Dixon, Kauffman & Neurath (1958). Fraction D3 gave only one strong ninhydrin-positive spot when tested by paper electrophoresis at pH 4 and by chromatography in butanol-acetic acid; the mobility at pH 4 was zero, and on chromatography its R_F values were less than that of cystine (i.e. less than any of the 'protein' amino acids). Acid hydrolysis (which was incomplete under the standard conditions) gave a basic amino acid whose R_F was lower than that of lysine, both in butanol-acetic acid-water-pyridine and in butanol-acetic acid. After electrophoresis in veronal, the ninhydrin-positive spot in D3 coincided with a molybdate-positive spot, and hydrolysis of D3 liberated orthophosphate as well as the amino acid, which was differentiated from lysine. These results suggested that D3 might be hydroxylysine phosphate. This was supported by the observation that (after electrophoresis in veronal) the amino acid spot in the hydrolysate of D3 reacted with periodate, although unhydrolysed D3 did not react.

There was no separation in mixed spots of hydroxylysine and the amino acid in the hydrolysate of D3 on chromatography in butanol-acetic acid, or on cation-exchange paper; the latter gave the best separation of lysine and hydroxylysine.

The two 'slow' spots, D5 and D6, in Fig. 1 were probably taurine and phosphorylethanolamine (2-aminoethyl phosphate); evaporation during the electrophoresis carries these substances nearly up to the apex of the paper. The other substances were present at low concentrations, and have not been well characterized. Both D1 and D2 were decomposed on acid hydrolysis, and both gave several ninhydrin-positive products.

Constituents of fraction 'E'. Fraction E was analysed by paper chromatography in butanol-acetic acid-water-pyridine (Fig. 2). The main constituents were glutamic acid, ophthalmic acid (Waley, 1956, 1958) and norophthalmic acid (Waley, 1957). The presence of the two peptides was confirmed by chromatography of hydrolysates. An unknown substance (E5) was also detected; its R_F was slightly greater than that of GSSG. The mobility of E5 on electrophoresis at pH 4 was similar to that of glutamic acid, and considerably less than that of GSSG. The new substance, E5, was thus not GSSG; GSSG was eluted from the ion-exchange column later (cf. peak G in Fig. 1 of Calam & Waley, 1963). That E5 was related to GSSG was suggested by the liberation on hydro-

Fig. 1. Electrophoresis of fraction D on no. 3 paper for 190 min. at 12 v/cm. in formic acid-acetic acid-water.

lysis of glutamic acid, cystine and glycine. Oxidation with performic acid gave comparable amounts of the sulphonic acid (GSO_3H) derived from glutathione, and of the sulphonic acid (CySO_3H) derived from cysteine. This result suggests that E5 is the mixed disulphide (CyS-SG) from cysteine and glutathione; this compound is present in extracts of pig liver (Plaquet, Biserte & Boulanger, 1962) and of guinea-pig liver (Neish & Rylett, 1963).

*Fractionation of lens extracts by
continuous electrophoresis*

A ninhydrin-positive substance was isolated by continuous electrophoresis at pH 4 whose mobility was intermediate between the neutral and basic amino acids. The material was first obtained from extracts of ox lenses, but more work has been done on the fractions (9–16, Expt. 1) from calf lenses obtained during the isolation of *S*-($\alpha\beta$ -dicarboxyethyl)glutathione (Calam & Waley, 1963). Calam (1962) suggested that this substance might have the structure arginyl-lysylglycine. This is now known

not to be so. Synthetic arginyl-lysylglycine (Walshaw & Young, 1964) and the material from the lens differed greatly in their electrophoretic mobilities. Although considerable evidence was originally obtained that the material from the lens was a basic peptide, even this is now uncertain as later attempts to prepare more of the material were unsuccessful.

An unidentified amine was also detected; its main characteristics were that it had an electrophoretic mobility at pH 4 comparable with the basic amino acids, and that it travelled near the solvent front on paper chromatography in several solvents. The substance was isolated from the fraction (tubes 5–8) containing the basic amino acids obtained by continuous paper electrophoresis of calf-lens extracts (Expt. 1 of Calam & Waley, 1963). This fraction (0.5 ml., equivalent to 30 g. of calf lens) was applied as a narrow band (15 cm. long) to no. 3 paper, and the paper developed in butanol-acetic acid for 8 hr. The ninhydrin-positive component (located by guide strips) with R_f 0.9 was eluted from the paper by 1% acetic acid.

The eluate gave a single ninhydrin-positive spot, with R_f 0.94 in phenol-ammonia, 0.90 in butanol-acetic acid and 0.89 in butanol-acetic acid-water-pyridine. The substance was stable to acid hydrolysis, and did not react with the Sakaguchi, Pauly or Ehrlich reagents, but was partially decomposed by nitrous acid.

Small quantities of a picrate were isolated by adding 15 μl . of saturated aq. picric acid to 100 μl . of about 10 mM solution of the base. The yellow crystals gave an infrared spectrum (as a potassium bromide disk) in which the strongest bands coincided with those of picric acid, but two bands at 3.4 and 6.75 μ (absent from picric acid) may be C-H stretching and C-H deformation bands due to CH_3 groups.

The base was not detected in an 'extraction' carried out without lenses; this was done to test whether the amine was a constituent of any of the reagents used (especially the *N*-methyldioctylamine). The concentration of the amine in the lens was probably about 0.04 mM.

The structure of this base is not known. It has a higher R_f than pentylamine, and so presumably has a longer carbon chain (dodecylamine had R_f 0.91 in butanol-acetic acid). No similar compound is included in the extensive list of amines given by Blau (1961), but Perry & Schroeder (1963) detected an amine with similar R_f values in urine.

*Analysis of lens extracts by
ion-exchange chromatography*

One fresh ox lens (2.1 g.) was ground under 20 ml. of 5% (v/v) formic acid and the mixture then centrifuged at 10000g at 0°. The supernatant

Fig. 2. Chromatography of fraction E in butanol-acetic acid-water-pyridine on no. 4 paper for 16 hr.

Table 1. *Amino acids in ox lens*

Preparation of the extract is described in the text. Amino acids given below are those that formed well-resolved peaks in the procedure of Spackman, Stein & Moore (1958).

Amino acid	Amount (mg./100 g. of lens)	Vol. of effluent (ml.)
Taurine	13.4	70
Urea	7.5	80
Ophthalmic acid	31.8	95
Aspartic acid	0.6	129
Threonine	1.4	140
Glutamic acid	20.0	215
Glycine	9.1	259
Alanine	7.1	270
α -Amino- <i>n</i> -butyric acid	0.6	305
Valine	3.1	338
Isoleucine	1.4	465
Leucine	2.2	481
Phenylalanine	1.7	567
Ornithine	1.1	182
Ammonia	0.9	227
Lysine	1.8	240
Histidine	0.2	350

was allowed to flow on to a column (31 cm. $\times$ 2.8 cm.) of Sephadex G-25 (equilibrated with 5 % formic acid) and eluted with the 5 % formic acid. The first 40 ml. of eluate was rejected, and then 5 ml. fractions were collected. The fractions were tested by putting spots on paper and developing with ninhydrin. Fractions 6–14 contained protein; fractions 21–35, which contained low-molecular-weight compounds, were pooled and concentrated on a rotary evaporator. Water was added and removed by distillation several times, to remove most of the formic acid. The mixture was then dried overnight in a vacuum desiccator. The residue, in 2 ml. of water, was brought to pH 8.8 with 4 drops of *N*-sodium hydroxide, and stood at room temperature overnight so that GSH should become oxidized. The nitroprusside test was now negative and the solution was evaporated *in vacuo*. Analysis by the method of Spackman, Stein & Moore (1958) (for which we are very grateful to Dr S. Moore) gave the results shown in Table 1. The value for ophthalmic acid is a maximum, since another peak partly overlapped. Other compounds that did not form well-resolved peaks (e.g. GSSG, glutamine, asparagine and serine) are not listed, since accurate values for the amounts present cannot be given. Other compounds probably present, but not listed in Table 1 owing to their low concentrations, include proline, methionine, tyrosine and arginine. There were also peaks present in the positions of glycerophosphorylethanolamine, phosphorylethanolamine and cystathione. Unidentified peaks were eluted from the 150 cm. column at the following volumes (ml.): 38, 41, 53, 63, 459.

Analysis of lens extracts by paper electrophoresis and chromatography

Since most of the ninhydrin-positive spots on chromatograms of extracts of tissues are due to amino acids, some study of the systems that separate amino acids was made. The best results were obtained by electrophoresis in formic acid-acetic acid, followed by chromatography in butanol-acetic acid in the second dimension. This system has been applied to the amino acids and amines in cerebrospinal fluid (Knauff, Mialkowsky & Zickgraf, 1959; see also Michl, 1961), and a closely similar system has been used for the amino acids in the hydrolysates of proteins (Richmond & Hartley, 1959). These authors used electrophoresis at high voltages (e.g. 100 v/cm.), which improves the separations. Nevertheless, even with low-voltage gradients (8–16 v/cm.) this system is very satisfactory. The experiments described below, illustrated in Calam (1962), were carried out on 46 cm. $\times$ 25 cm. sheets of no. 3 paper; electrophoresis was carried out first for 3.5 hr. (in the short direction) followed by chromatography in the long direction. The pattern of ninhydrin-positive spots is referred to as a 'map'.

Continuous electrophoresis of lens extract at pH 4 (Expt. 1 of Calam & Waley, 1963) gave a series of fractions, and maps of these were first studied, as they were simpler than those from unfractionated extracts. Moreover, knowledge of the mobility at pH 4 helped to predict what substances would be present.

Calf-lens extracts. The lens (0.9 g.) was ground under cooled 7 % (w/v) trichloroacetic acid (2 ml.) and the mixture clarified by centrifuging. The supernatant was extracted with *N*-methyldiethylamine in chloroform (3.2 ml. of 5 %, v/v) in several portions; the layers were separated by centrifuging and the aqueous layer was evaporated *in vacuo*. The residue (14 mg.) was dissolved in water (20 μ l.) and 10 μ l. taken for the map.

Protein may also be removed with phenol (Calam & Waley, 1963), which avoids strongly acidic conditions; the map from this extract was very similar to that obtained from trichloroacetic acid extracts, and the latter method was preferred.

Amino acids and related compounds in hydrolysed extracts. The map from hydrolysed extracts is simpler than that from unhydrolysed extracts, and so is discussed first.

The following 17 protein amino acids were readily identified (the numbers refer to those in Fig. 2 of Calam, 1962): cystine (5), lysine (9), histidine (10), arginine (12), glycine (14), serine (15), aspartic acid (16), threonine (17), glutamic acid (18), alanine (20), proline (23), tyrosine (27)

methionine (29), valine (30), phenylalanine (31), leucine (32) and isoleucine (33); tryptophan, asparagine and glutamine are absent from hydrolysates. Phosphorylethanolamine (6) was detected; it is rather resistant to hydrolysis (Tallan, Moore & Stein, 1954). Hydroxylysine (2), cystathione (7), ornithine (8), 1-methylhistidine (11), taurine (13), *S*-($\alpha\beta$ -dicarboxyethyl)cysteine (19), ethanolamine (21), β -alanine (22), γ -aminobutyric acid (25) and α -aminobutyric acid (26) make up another 11 compounds. Of these, hydroxylysine, cystathionine, ornithine, 1-methylhistidine, *S*-($\alpha\beta$ -dicarboxyethyl)cysteine and γ -aminobutyric acid were not reported by Dardenne & Kirsten (1962), who analysed acid hydrolysates of extracts of calf lens by ion-exchange chromatography; their unknown amino acid between valine and methionine might be cystathionine. On the other hand, they report the presence of hydroxyproline, sarcosine, glucosamine and phosphoserine. The peak ascribed to phosphoserine may be due to cysteic acid, which is probably the substance responsible for the spot 46 in Fig. 2 of Calam (1962). There remain six unidentified spots in Fig. 2. Two of these (3 and 24) gave yellow colours with the ninhydrin reagent used (containing cobalt chloride) and are probably not amino acids; one (66) was detected also in hydrolysates of the acidic fractions obtained by continuous paper electrophoresis.

Of the compounds not reported by Dardenne & Kirsten (1962), cystathionine, ornithine, 1-methylhistidine and γ -aminobutyric acid were detected (by ion-exchange chromatography) in rabbit lens by Reddy & Kinsey (1962). The latter two were present at low concentrations. Ornithine and 1-methylhistidine were present in the fractions obtained by continuous paper electrophoresis that contained the basic amino acids. Their presence in these fractions was established by electrophoresis at pH 4, and in borate buffer, and by chromatography in butanol-acetic acid, in phenol-ammonia (Reddy & Hegsted, 1962), and ornithine was also separated from lysine in butanol-acetic acid-water-pyridine. γ -Aminobutyric acid and β -alanine were detected in the fractions obtained by continuous electrophoresis that contained the basic peptide described above; their identity was established by chromatography in several solvents. Hydroxylysine is a hydrolysis product of hydroxylysine phosphate (see above), and *S*-($\alpha\beta$ -dicarboxyethyl)-cysteine has been identified previously (Calam & Waley, 1963).

Amino acids and related compounds in unhydrolysed extracts. Nearly all the compounds present in hydrolysed extracts were also detected in unhydrolysed extracts, together with many additional compounds (Fig. 1 of Calam, 1962). Of the protein amino acids, asparagine (50), glutamine (53) and

tryptophan (63) were now present. The characteristic blue fluorescence of tryptophan in ultraviolet light was helpful in comparing maps. Citrulline is probably present since the neutral fraction (from continuous paper electrophoresis) gave a spot reacting with Ehrlich's reagent in the expected position. This is the position (53) occupied by glutamine, so the ninhydrin-positive spot in the extract in this position is probably due to both glutamine and citrulline. This spot is much weaker in extracts from stored lenses as the glutamine has cyclized to 2-oxopyrrolidine-5-carboxylic acid. This substance was isolated from the most acidic fraction (tubes 30-32) obtained by continuous electrophoresis of extracts of stored lens. A portion of this fraction was submitted to preparative paper chromatography in butanol-acetic acid, and the band with the expected R_F value eluted and shown to liberate glutamic acid on hydrolysis.

Of the non-protein amino acids, the only one unstable to hydrolysis was hydroxylysine phosphate (41) (the spot immediately above spot 42 in Fig. 1 of Calam, 1962, should be numbered 41, not 40); another amino compound unstable to hydrolysis was glycerophosphorylethanolamine (49). Both these substances were molybdate-positive as well as ninhydrin-positive.

No neutral peptides have been identified. Carnosine (47), a basic peptide, was isolated from ox lenses (Krause, 1936); its presence in calf lens is inferred from work on the fraction obtained by continuous paper electrophoresis that contained the basic amino acids. Carnosine was detected as a ninhydrin-positive (grey colour) and Pauly-positive spot after electrophoresis, or chromatography in butanol-propanol-ammonia for 49 hr. on no. 3 paper; this fraction also liberated β -alanine on hydrolysis.

Of the acidic peptides, GSSG (42) was readily identified, but the others occupied a rather congested area of the map, which was affected by distortion of the spots at the apex of the paper. Most of the glutathione was in the oxidized form by the time that paper chromatography was carried out; there is little difference between the mobilities of GSH and GSSG in the formic acid-acetic acid buffer, so that a mixed spot (of GSH and GSSG) will be present after electrophoresis. When the paper is dried in a draught of air, most of the remaining GSH would be expected to be oxidized. Hence, after chromatography, the main spot will be in the position of GSSG. *S*-Sulphoglutathione (48) (Waley, 1959) was one of the acidic substances with a low R_F . *S*-($\alpha\beta$ -Dicarboxyethyl)glutathione (54) (Calam & Waley, 1963) and ophthalmic acid (58) (Waley, 1956, 1958) were identified with markers; norophthalmic acid (Waley, 1957) may also be present in spot 54.

A spot (62) which gave an atypical ninhydrin colour (initially purple with the reagent containing cobalt chloride), and which quenched the background fluorescence of the paper in ultraviolet light, was identified as ascorbic acid. The colour yield on paper is only about one-tenth of that of an amino acid, but ascorbic acid is relatively abundant in lens. Authentic ascorbic acid was detected (on paper) by the ninhydrin reagent containing cobalt chloride, and by 0.2 % ninhydrin in acetone, but not by ninhydrin (0.5 %) in acetone containing 5 % (v/v) of water and 5 % (v/v) of acetic acid. The ninhydrin reagent does not usually detect non-nitrogenous compounds; another such non-nitrogenous compound is laevulinic acid, present in hydrolysates of plant extracts (Zacharius & Talley, 1962).

Some compounds detected by other reagents. Attention has been mostly confined to the identification of ninhydrin-positive compounds, but some other reagents have been used, mostly as ancillaries to the ninhydrin reagent. A strong spot was given by urea by the Ehrlich reagent. Molybdate-positive spots were attributed to phosphorylcholine, glycerophosphorylcholine and α -glycerophosphate, which occur at quite high concentrations in lens (van Heyningen & Pirie, 1958). Inositol, glucose and creatine have also been tentatively identified. Ergothioneine, which has not previously been detected in lens, was identified by its quenching in ultraviolet light, and by its reaction with the Pauly reagent. The concentration of ergothioneine in lens is about 0.1 mM. Several unidentified spots were detected with the Pauly reagent; one may be hercynine (histidine-betaine) which is present in cattle blood (Ackermann, Litz & Menssen, 1959).

Unidentified ninhydrin-positive compounds. Three spots (43, 55 and 59) appear near the apex of the paper, and it is uncertain whether they are caused by streaking of spots 42, 54 and 58. They are absent from the hydrolysate. There are about 12 other unidentified spots; their common characteristics are that they are relatively faint, absent from hydrolysates and were detected in the acidic fractions obtained by continuous electrophoresis. Whether they are acidic peptides remains to be determined.

Ox lens. Extracts from ox lens were examined before and after hydrolysis, and were very similar to those from calf lens.

Human (cataractous) lens. The lenses (4.1 g.) were ground with water (8 ml.) and then dialysed against 120 ml. of water (saturated with chloroform) at 4° for 3 days. The outer liquid was replaced and dialysis continued for 4 days. The diffusates were combined, the water was removed by distilling under reduced pressure, and the residue dissolved

in 0.4 ml. of water to give a yellow alkaline solution. Although the general pattern given by this extract was similar to that obtained from cattle lens, there were several differences. The most striking difference was the fluorescent peptide, detected in the lens of primates by François, Rabaey & Recoulès (1961). The concentration of this substance seemed comparable with that of the less abundant amino acids. There were again several unidentified substances, some of which seemed to be the same as in the calf lens extract, and some of which were different. There were fewer unidentified compounds in the hydrolysate, but there was one (apparently present in relatively large amount) which was only partially separated from aspartic acid. The amount of aspartic acid in the hydrolysate was also unexpectedly large, as there was relatively little asparagine before hydrolysis.

DISCUSSION

Fractionation of lens extracts. Since it is not possible to plan a procedure for separating unknown compounds from the 50 or so known compounds, the new compounds that are detected tend to have some unusual chromatographic or electrophoretic properties. During development of a method for isolating *S*-($\alpha\beta$ -dicarboxyethyl)glutathione by ion-exchange chromatography (Calam & Waley, 1963) a small peak (D) was observed, which preceded the known acidic compounds. The major constituents of this fraction have now been identified as taurine, aminoethyl phosphate and hydroxyllysine phosphate. The last has not often been reported as a constituent of tissue extracts, although its presence in calf embryo and in some other tissues has been noted (Gordon, 1948; Astrup, Carlström & Stage, 1952). Minor constituents of this fraction are present in too small amounts for further study. Oligopeptides might be present, as they are retarded on anion-exchange columns, relative to amino acids, although for this separation a buffer with a higher pH than the one (5.5) used is preferable (Carnegie, 1961). It seems unlikely that simple oligopeptides (other than γ -glutamyl peptides) are present except at very low concentrations, since peptidases are active in lens, and protein breakdown does not result in the accumulation of oligopeptides (van Heyningen & Waley, 1963). The mixed disulphide (CyS-SG) from cysteine and glutathione, isolated from calf lens extracts, may exist as such in the lens, or it may be formed during the course of extraction and fractionation. This mixed disulphide is present in extracts of pig liver, but not in extracts of sheep liver (Plaquet *et al.* 1962).

A compound detected owing to its unusual behaviour on paper chromatography may be a long-

chain amine. Scarcity of material has prevented a thorough investigation.

Analysis of lens extracts. The method of paper electrophoresis, followed by paper chromatography, used in this work has proved useful in demonstrating the presence of a number of amino compounds in the lens. The resolving power of this system is nearly comparable with that of ion-exchange chromatography (Spackman *et al.* 1958), and the apparatus is simpler. Certain labile compounds, notably tryptophan and glutamine, are more readily detected on paper. For accurate quantitative data, on the other hand, ion-exchange chromatography is greatly superior to paper chromatography. The values given in Table 1 for ox lens may be compared with those obtained by Reddy & Kinsey (1962) for rabbit lens. There are numerous individual differences, but a general feature is that the amino acids appear to be present in larger amounts in rabbit lens. It should be noted that different methods were used to separate amino acids from proteins (picric acid in one case, gel filtration in the other), and it is possible that this could affect the results.

Methodological differences also complicate comparison of the results in Table 1 with those obtained by Dardenne & Kirsten (1962), who analysed acid hydrolysates of perchloric acid extracts of ox lens. Ox lens protein is not completely precipitated by perchloric acid, and the amount of protein (about 65 mg./100 g. of lens; N. J. Pritchard, personal communication) in the supernatant is sufficient to give appreciable amounts of amino acids after acid hydrolysis.

SUMMARY

1. Extracts of calf lens have been fractionated by ion-exchange chromatography and continuous paper electrophoresis. The fractions have been studied by paper electrophoresis and paper chromatography.

2. The phosphate of hydroxylysine, and the mixed disulphide derived from cysteine and glutathione, have been isolated. Most of the other ninhydrin-positive low molecular-weight-compounds have been identified by paper electrophoresis at pH 1.6, followed by paper chromatography.

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