of the cations. The effect of some thiol reagents is diminished by activators (K^+ and Rb^+ ions) and increased by inhibitors (Na^+ and Li^+ ions). There is no general correlation between the effect of ions on the enzyme activity and the reactivity of the thiol groups. Cations which activate the TPN-specific enzyme do not affect significantly the enzyme inhibition by thiol reagents.

6. Some convenient modifications have been introduced in the preparation of both yeast aldehyde dehydrogenases.

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Reaction of Ninhydrin in Acid Solution with Straight-Chain Amino Acids Containing Two Amino Groups and its Application to the Estimation of $\alpha\epsilon$ -Diaminopimelic Acid

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Certain basic amino acids, such as ornithine and lysine, were reported by Chinard (1952) to give coloured products when heated with ninhydrin in solution at about pH 1. With the exception of proline, the other common naturally occurring amino acids did not react significantly under these conditions. Since lanthionine also reacted (Newton, Abraham & Berridge, 1953) it was of interest to examine $\alpha\epsilon$ -diaminopimelic acid, which is structurally related to both lanthionine and lysine and which is widely distributed among many species of bacteria (Work & Dewey, 1953; Hoare & Work, 1957). The product of reaction of acidic ninhydrin with diaminopimelic acid was yellow and thus resembled the reaction products from lysine or lanthionine, but differed from the red product given by ornithine.

Other similar amino acids containing two amino groups are the various naturally occurring sulphur-

containing diaminodicarboxylic acids and also the higher and lower homologues of diaminopimelic acid. Their reaction products with acidic ninhydrin were all examined in order to determine whether the reaction is a general one for diamino acids. The reaction of acidic ninhydrin with diaminopimelic acid and with certain other naturally occurring amino acids has been investigated with a view to finding a specific method for estimating diaminopimelic acid.

MATERIALS AND METHODS

Unless otherwise stated, diaminopimelic acid and its homologues were unresolved synthetic samples; I am indebted to Dr H. Heath for diaminosuccinic and diaminoglutaric acids and to Dr D. Simmonds for other members of the homologous series. *S*-(β -Aminoethyl)cysteine was provided by Dr H. Lindley. *LL*- and *meso*-Diaminopimelic acid were prepared as described by Hoare & Work (1955). The other

amino acids were commercial samples. Basic and diamino-dicarboxylic amino acids were used in aqueous solutions in concentrations up to 3 mM, depending on the optical density of the reaction products; other neutral and acidic amino acid solutions were 50–100 mM.

Ninhydrin reagents. These were based on that described by Chinard (1952). Ninhydrin (recrystallized or A.R. grade, 250 mg.) was dissolved in acetic acid (A.R. grade, 6 ml.) and the solution mixed with 4 ml. of (a) aq. 6M-phosphoric acid, or (b) aq. 0.6M-phosphoric acid, or (c) water. These mixtures are referred to as reagents (a), (b) and (c). Reagent (d) was made by dissolving 250 mg. of the ninhydrin in 10 ml. of the acetic acid.

Conditions for the standard reaction. The amino acid solution (0.5 ml.) was mixed with acetic acid (0.5 ml.) and ninhydrin reagent (0.5 ml.). The resulting reaction mixture was at pH 0.3, 0.9 or 1.0 according to the concentration of phosphoric acid (a, b or c above). The solutions were covered and heated in a bath at 100° for the stated times, removed from the bath and cooled rapidly to room temperature. Acetic acid (3.5 ml.) was added to bring the final volume to 5 ml. A reagent blank containing water instead of the amino acid solution was treated identically. Any further dilution was with acetic acid, the reagent blank also being diluted.

After the findings on cyclic imino acids by Schweet (1954) and Piez, Irreverre & Wolff (1956), modifications to the standard reaction were investigated. The reaction was carried out in a volume of 5 ml., all the acetic acid (4 ml.) being added before the ninhydrin reagent. The total amounts of water present were 14% (the usual amount) or 2%. In the latter case ninhydrin reagent (d) was used and the amino acid sample was added in 0.1 ml. of water.

The optical density of the solutions of the various wavelengths were read against the reagent blank in 1 cm.³ silica cells in a Unicam spectrophotometer, type SP. 500, or (at 440 m μ) in type SP. 600.

RESULTS

Reaction of acidic ninhydrin with amino acids containing two amino groups

The reaction between diaminopimelic acid and acidic ninhydrin was first carried out in the mixture used by Chinard (1952), namely ninhydrin reagent (a) made from 6M-phosphoric acid (final pH, 0.3) and 1 ml. of all reactants. A yellow reaction product was produced and the spectrum showed absorption peaks of approximately equal intensity at 347 and 430 m μ (cf. Fig. 1, curve A). When a less acidic reaction mixture (b, pH 0.9) was used, the spectrum (cf. curve B) had a single peak at 347 m μ ; absorption at 430 m μ was reduced by about 50%. The spectrum of the lysine-reaction product published by Chinard (1952) (see also curve C) resembled that produced from diaminopimelic acid at pH 0.9 except that lysine gave more absorption above 500 m μ ; with lysine, change in acidity had no effect on the spectrum.

The extinction coefficients of the diaminopimelic acid reaction product were reproducible at either

345 or 440 m μ when the ninhydrin reaction was carried out at pH 0.9, but at pH 0.3 much greater variations from one experiment to another were obtained, although duplicates from the same experiments agreed well. A pH of 0.9 was therefore chosen for investigation of the reaction products from other amino acids.

Since the reaction mixture described by Chinard (1952) contained ninhydrin in great excess over the amino acid, the contribution of the unused ninhydrin to the spectrum was investigated. The spectrum, read against water, of a blank mixture made from 1 ml. of ninhydrin reagent (b), 1 ml. of water and 1 ml. of acetic acid, heated for 1 hr. and diluted to 5 ml., showed very strong absorption at 320–340 m μ ($E=2.0$) with the peak at less than 320 m μ ; above 430 m μ there was no absorption. The unreacted ninhydrin thus contributed considerably to total absorption at 345 m μ , consequently any errors introduced during pipetting of the ninhydrin reagent would be reflected in the final absorption at 345 m μ . Comparisons were made of reaction products from several amounts of

Fig. 1. Absorption spectra of products obtained by heating amino acids with acidic ninhydrin at 100° for times shown. Other conditions were as described in Methods. A, Diaminopimelic acid heated for 5 min.; ninhydrin reagent (a); B, diaminopimelic acid heated for 5 min., ninhydrin reagent (b); C, lysine heated for 60 min., ninhydrin reagent (b); D, diaminoadipic acid heated for 60 min., ninhydrin reagent (b).

Table 1. *Light-absorption characteristics of products from reaction of ninhydrin at pH 0.9 with amino acids containing two amino groups*

Amino acid (0.2-3 mm) 0.5 ml., heated with 0.5 ml. of acetic acid and 0.5 ml. of ninhydrin reagent (b) [2.5%, w/v, solution in acetic acid-0.6M-phosphoric acid (3:2)]. Reaction temperature was 100°; unless otherwise stated reaction time was 5 min., after which mixtures were diluted to 5 ml. with acetic acid.

Amino acid	Type	Atoms separating amino groups	Final concn. (μmoles/5 ml.)	Main peak			Other peaks			$\frac{E_{\text{max.}} (80 \text{ min.})^*}{E_{\text{max.}} (5 \text{ min.})}$
				$\lambda_{\text{max.}}$ (mμ)	$E_{\text{max.}}$ (observed)	$E_{\text{max.}} \times 100$ (calc./μmole)	$\lambda_{\text{max.}}$ (mμ)	$E_{\text{max.}}$ (observed)	$\frac{E_{840 \text{ mμ}}}{E_{440 \text{ mμ}}}$	
Diaminosebacic acid	Neutral	C ₈	3.0	330-370	0.110†	3.7	—	None	—	—
Diaminoazelaic acid	Neutral	C ₇	3.0	330	0.275†	9.2	—	None	—	—
Diaminosuberlic acid	Neutral	C ₆	1.0	325	0.595†	59.5	—	None	2.0	0.8
Diaminopimelic acid	Neutral	C ₅	0.6	345	0.960	160	—	None	2.1	0.95
Lysine	Basic	C ₅	0.7	340	1.002†	143	—	None	2.1	1.3
Diaminoadipic acid	Neutral	C ₄	0.2	515	0.850	425	{ 480 370 }	{ 0.600 0.310 }	—	1.1
Ornithine	Basic	C ₄	0.2	515	0.830	415	{ 475 370 }	{ 0.610 0.380 }	—	1.1
Diaminoglutaric acid	Neutral	C ₃	0.6	325	0.720	120	—	None	3.4	0.97
αγ-Diaminobutyric acid	Basic	C ₃	3.0	330	0.770†	25.6	—	None	4.0	—
Diaminosuccinic acid	Neutral	C ₃	3.0	330	0.410†	13.7	—	None	—	—
Homocysteine	Neutral	C ₃ ·S ₂ ·C ₃	3.0	325	0.495†	16.5	—	None	4.2	—
Djenkolic acid	Neutral	C ₃ ·S·C·S·C ₃	1.5	360	0.800†	53.3	460	0.400	2.7	11.8
Cystine	Neutral	C ₃ ·S ₂ ·C ₃	0.5	345	0.660	130	—	None	20.6	0.6
Cystinamine	Basic	C ₃ ·S ₂ ·C ₃	3.0	335	0.305†	10.2	—	None	17.0	5.5
Cystathionine	Neutral	C ₃ ·S·C ₃	0.4	340	0.600	150	—	None	28.0	1.0
Lanthionine	Neutral	C ₃ ·S·C ₃	0.2	360	0.660	330	450	0.422	1.2	0.5
S-(β-Aminoethyl)cysteine	Basic	C ₃ ·S·C ₃	0.2	340	0.900	450	440§	0.280§	8.3	0.4

* Ratio of extinction at $\lambda_{\text{max.}}$ of main peak after heating for 60 min. to that after heating for 5 min.

† 60 min. heating time.

‡ 35 min. heating time.

§ Apparent as a peak only after heating for 60 min.

diaminopimelic acid, and 1.0, 0.5 or 0.25 ml. of ninhydrin reagent (b), with corresponding reductions in the volumes of acetic acid and water so as to equalize the proportions of the components of the reaction mixture. Final dilution was 5 ml. in each case. The only effect of lowering the amount of ninhydrin was to reduce peak absorption at the shorter wavelengths and so define the peak at 345 m μ ; 0.5 ml. of all reactants was chosen as a convenient volume.

The reaction of ninhydrin reagent (b) with all available homologues of diaminopimelic acid and lysine was investigated, after various times of heating (Table 1). The reaction with diaminopimelic acid was complete after heating for 2 min.; heating for 60 min. reduced the absorption slightly compared with that after 5 min. (Table 1, column 11). With lysine the reaction was not complete after 5 min. The homologous diaminodicarboxylic acids, containing two to six carbon atoms in the chain, reacted strongly; diaminoadipic acid (Fig. 1, curve D) gave a red product almost identical with that from ornithine ($\lambda_{\max}$, 515, 475, 370 m μ); the products from the other amino acids were yellow and

all had similar types of absorption spectra with one peak between 325 and 345 m μ . The sulphur-containing diaminodicarboxylic acids also reacted under these conditions (Table 1 and Fig. 2). Cystine, cystathionine and homocystine after 5 min. heating gave colourless products with single absorption peaks between 325 and 340 m μ and low absorption beyond 440 m μ ; they differed from the non-sulphur-containing compounds in having low absorption at wavelengths longer than 400 m μ (see Table 1, column 10). With cystine (Fig. 2, curves C), after heating for 60 min. the peak was lower and absorption above 400 m μ had increased, and the colour changed to pale pink. Lanthionine, after 5 min. heating, produced a yellow product with a strong peak at 360 m μ and a subsidiary peak at 450 m μ ; heating for 60 min. lowered the absorption at 360 m μ only so that the main peak was then at 450 m μ (Fig. 2, curves A). Djenkolic acid did not react after 5 min.; heating for 30 min. produced a product with peaks at 360 and 450 m μ , but after 60 min. absorption at both peak wavelengths was reduced.

Basic amino acids reacted to give products having spectra similar to those of their neutral counterparts, but generally with lower extinction coefficients. An exception was *S*-(β -aminoethyl)cysteine. Here the colourless product produced by heating for 5 min. had a spectrum (Fig. 2, curve B) with a very high peak at 340 m μ , and thus resembled cystine rather than lanthionine. The effect of longer heating was to lower the absorption at 340 m μ and raise it at 440 m μ so that a second peak was produced.

Lowering the pH of the reaction mixture to 0.3 had little effect on the shape of the spectrum of the reaction products with the exceptions of diaminopimelic acid (already discussed) and of djenkolic acid and lanthionine where the optical density at the shorter wavelength peaks was decreased and that at the longer wavelength peaks increased. With other amino acids, there was sometimes no significant effect, or else the main peak was changed in intensity without change in position.

The extinction coefficients under the various conditions of test were not always reproducible, particularly with the bases and the sulphur-containing compounds. This was probably because the reaction conditions chosen were not optimum.

Reaction of acidic ninhydrin in the estimation of diaminopimelic acid

The standard reaction at pH 0.9 (i.e. with ninhydrin reagent b) and 5 min. heating gave a linear relationship between optical density at 345, 340 or 440 m μ and the amount of diaminopimelic acid added within the range 0.03–0.6 μ mole (6–115 μ g.), Fig. 3. Measurements at 440 m μ could be made in a photoelectric colorimeter, e.g. Unicam SP. 600,

Fig. 2. Absorption spectra of products obtained by heating sulphur-containing amino acids with ninhydrin reagent (b). Curves A, lanthionine: —, heated for 5 min.; ---, heated for 35 min. Curves B, *S*-(β -aminoethyl)-cysteine: —, heated for 5 min.; ----, heated for 60 min. Curves C, cystine: —, heated for 5 min.; ---, heated for 60 min.

although sensitivity and range were decreased. Further dilution with acetic acid of those solutions giving too strong absorption resulted in readings which did not lie on the same straight line as the undiluted solutions (see dotted line, Fig. 3). The *meso* and *LL* isomers of diaminopimelic acid reacted identically (Hoare & Work, 1957).

In order to assess the suitability of this reaction for the estimation of diaminopimelic acid in biological materials, the ninhydrin reaction products of all other naturally occurring amino acids were tested under these conditions for absorption at 340 and 440 $m\mu$. With the exception of the amino acids included in Table 1, only cystine, tryptophan, tyrosine, histidine and proline gave ninhydrin products with significant absorptions at these wavelengths (Table 2). The other amino acids, tested at levels of 10 μM /5 ml. or over, gave $E_{340\ m\mu}$ less than 0.3 and $E_{440\ m\mu}$ less than 0.1. Ammonia in amounts up to 120 μg /5 ml. did not give a coloured product but it apparently reacted with the ninhydrin, as 30 μg . or more caused decreases in absorption at 340 $m\mu$. The presence of both alanine and glutamic acid, which often accompany diaminopimelic acid in bacterial peptides (Work,

Fig. 3. Effect of diaminopimelic acid concentration on optical density at 345 and 440 $m\mu$ of the product of standard reaction with ninhydrin reagent (b) in 1.5 ml. for 5 min. at 100°, the mixture being diluted finally to 5 ml. with acetic acid. Other conditions of test were as in Methods. ●, Extinction at 345 $m\mu$; ▲, extinction at 440 $m\mu$; the dotted line joins points obtained after diluting the final reaction mixture (5 ml.) from 0.75 μmole of diaminopimelic acid with an equal volume of acetic acid.

1957), in concentrations ten times that of diaminopimelic acid had no effect on the result.

Attempts were made to find reaction conditions producing minimum interference from other amino acids without affecting reproducibility of diaminopimelic acid results. Table 2 shows some results from tests under various conditions on diaminopimelic acid and on the interfering amino acids likely to occur in significant concentrations in biological materials.

The standard reaction conditions were ultimately found to be the most satisfactory for diaminopimelic acid estimation. Although not applicable in the presence of lysine and certain other amino acids, these conditions are most convenient because of the short reaction time, wide range of diaminopimelic acid concentration and absence of interference by hydrochloric acid. When interfering amino acids cannot be eliminated and hydrochloric acid is not present, reaction in 5 ml. at 37° for 1.5 hr. can be used (Table 2), provided absorption is measured at 440 $m\mu$. Under these conditions diaminopimelic acid gave products with two absorption peaks at 355 and 430 $m\mu$; the strength of the phosphoric acid present (i.e. in ninhydrin reagents a, b or c) did not influence the reaction products, but the presence of hydrochloric acid prevented colour development. Lysine gave products with low absorption at 440 $m\mu$: but at the high lysine concentration of 3 $\mu\text{moles}/5\text{ ml.}$ an $E_{440\ m\mu}$ of 0.100 was obtained. Tyrosine at a concentration of 5.5 $\mu\text{moles}/5\text{ ml.}$ gave an $E_{440\ m\mu}$ of 0.260. Attempts to reduce the amount of water in the system to 2% of the volume, although giving colour specifically with diaminopimelic acid, led to results which varied from one experiment to another. With the usual proportion (14%) of water consistent results were obtained at 37°, although the straight-line relationship between optical density, measured at either peak, and diaminopimelic acid concentration persisted only up to 0.3 $\mu\text{mole}/5\text{ ml.}$ Some of the sulphur-containing diamino acids reacted strongly under these conditions, e.g. lanthionine (0.2 $\mu\text{mole}/5\text{ ml.}$, $E_{340\ m\mu}$ 0.432, $E_{440\ m\mu}$ 0.260), *S*-(β -aminoethyl)cysteine (0.2 $\mu\text{mole}/5\text{ ml.}$, $E_{340\ m\mu}$ 160, $E_{440\ m\mu}$ 110).

Diaminopimelic acid can be estimated directly by the standard method in biological materials, such as enzymic reaction mixtures, where it is present in high concentrations compared with other amino acids. For example, an experiment was carried out to find out whether diaminopimelic acid was metabolized anaerobically by an asporogenous variant strain of *Bacillus sphaericus* (N.C.T.C. 7582). Warburg flasks contained 30 mg. of acetone-dried cells in 1.6 ml. of 0.1 M-phosphate buffer, pH 6.8, and after gassing with nitrogen, 4 mg. of synthetic diaminopimelic acid in 0.4 ml. of water was tipped

Table 2. Extinction coefficients of acidic ninhydrin reaction products of some naturally occurring amino acids

Amino acid	Final concn. (μ mole/5 ml.)	Heating time (min.)	Reaction in 1.5 ml. at 100° at pH shown, final dilution to 5 ml.						Reaction in 5 ml. at 37° for 90 min.†	
			pH 0.3*		pH 0.9†		pH 1.0‡			
			$E_{340\text{ m}\mu}$	$E_{440\text{ m}\mu}$	$E_{340\text{ m}\mu}$	$E_{440\text{ m}\mu}$	$E_{340\text{ m}\mu}$	$E_{440\text{ m}\mu}$	$E_{340\text{ m}\mu}$	$E_{440\text{ m}\mu}$
Cysteine	0.8	5 60	— —	— —	0.245 0.135	0.015 —	— —	— —	— —	— —
Cystine	0.5	5 60	0.675 0.530	0.025 0.090	0.650 0.420	0.020 0.107	0.670 0.500	0.020 0.142	0 —	— —
Diaminopimelic acid	0.3	5 60	0.360-0.475 0.450-0.570	0.310-0.480 0.350-0.450	0.430-0.500§ 0.435-0.460	0.210-0.229§ 0.190-0.229	0.420 0.470	0.176 0.202	0.245 —	0.450 —
Histidine	5.0	5 40	— 0.695	— 0.285	0.355 0.820	0.036 0.260	— —	— —	0.550 —	0.040 —
Lysine	0.5	5 60	0.570 0.740	0.143 0.340	0.450 0.600	0.168 0.360	0.450 0.660	0.153 0.340	0.057 —	0.011 —
Ornithine	0.5	5 60	0.176 0.892	0.088 0.618	0.315 0.710	0.245 0.645	0.329 0.770	0.340 0.620	0.200 —	0 —
Proline	0.5	5 60	0.200 0.645	0.100 0.484	0.365 0.710	0.164 0.600	0.430 0.550	0.180 0.505	0.640 —	0.066 —
Tyrosine	5.5	5 40	— 1.070	— 0.200	0.590 0.580	0.072 0.096	— —	— —	1.150 —	0.260 —
Tryptophan	0.5	5 60	0.338 0.830	0.067 0.197	0.282 0.658	0.066 0.191	0.116 0.468	0.060 0.147	0.100 —	0.014 —

* Ninhydrin reagent (a).

† Ninhydrin reagent (b).

‡ Ninhydrin reagent (c).

§ Eight experiments, mean 462 and 222 respectively.

from the side arm. A control flask contained no added amino acid. After incubation for various times at 37°, each flask was heated for 5 min. at 100° and its contents were centrifuged; 0.5 ml. of the supernatant was added to 5 ml. of acetic acid. Diaminopimelic acid was estimated in 0.5 ml. of this mixture, 0.5 ml. of ninhydrin reagent (b) and 0.5 ml. of water being added. The standards consisted of the control flask similarly treated (0.5 ml.), to which were added 0.5, 0.4, 0.3 and 0.2 ml. of diaminopimelic acid (0.2 mg./ml.) and water to 0.5 ml. After 5 min. at 100° and dilution to 5 ml., absorption at 440 m μ was read, the contents of the control flask without added diaminopimelic acid being used as blank. The amounts of diaminopimelic acid in each flask after 0, 30, 60 and 300 min. incubation were 4.32, 4.40, 4.48 and 4.05 mg. respectively. This indicated an experimental error of about 10%, since it was evident that diaminopimelic acid had not been metabolized.

The method is suitable for estimation of diaminopimelic acid in acidic eluates from ion-exchange columns; for example, in preparing diaminopimelic acid from culture filtrates of mutant *Escherichia coli* 26-26, the culture filtrate (200 ml.) (Work & Denman, 1953) was passed through a column of Zeo-Karb 225 (4½% cross-linked) 60 cm. $\times$ 0.9 cm. with 1.25N-hydrochloric acid as eluent (Hoare & Work, 1955). The diaminopimelic acid peak was detected by testing 0.1 ml. samples of the eluate by the standard ninhydrin reaction (reagent b, vol. 1.5 ml.). Ammonia does not interfere with the reaction and no trouble was experienced with variations in base line. Diaminopimelic acid can also be estimated in 'spots' cut from paper chromatograms, as there are no blank reactions given by other parts of the filter paper (I am indebted to Mr R. E. Strange for this information). A straight-line relationship was found between colour at 440 m μ , which developed on heating the cut-out filter-paper 'spots' in the reaction mixture, and the amount of diaminopimelic acid (0.1-1.0 μ mole) applied to the chromatogram. Methanol-water-10N-hydrochloric acid-pyridine (80:17.5:2.5:10) was the solvent system for development of the chromatogram (Hoare & Work, 1955).

DISCUSSION

Ninhydrin reacts specifically under certain acidic conditions with all straight-chain amino acids containing two amino groups. It is also known to react with cyclic imino acids (Piez *et al.* 1956). As the dibasic amino acids behave in essentially the same way as their neutral dicarboxylic acid counterparts, evidently only one amino group has to be in the position α to a carboxyl group. Since neither putrescine nor cadaverine reacts (see also Chinard,

1952) one carboxyl group is essential. All members of the homologous series of diaminodicarboxylic acids with less than seven carbon atoms separating the amino groups react strongly, and longer chain homologues react weakly. With the exception of diaminoadipic acid (C₄ chain), the distance between the amino groups has little effect on the spectrum of the reaction products, which has a single absorption maximum near 340 m μ . The red product from diaminoadipic acid is the same as that described by Chinard (1952) for ornithine and proline. Calculation of optical densities at the absorption maxima near 340 m μ on an equimolar basis shows that there is a maximum for diaminopimelic acid (C₅ chain). Under more acidic conditions, the product from diaminopimelic acid shows a second absorption peak near 440 m μ .

The sulphur-containing diamino acids give products with stronger absorption than their carbon homologues; in fact, the product from *S*-(β -aminoethyl)cysteine has the highest optical density at 340 m μ of all the compounds investigated. Under certain reaction conditions the products from some of the sulphur-containing compounds show a second peak near 450 m μ ; this is the wavelength given by Newton *et al.* (1953) for the lanthionine peak. Cysteine has been reported by Chinard (1952) to give a coloured product and this has been confirmed for a freshly prepared solution; it is possible, however, that cysteine may only react after oxidation to cystine, since the reaction seems to be specific for diamino compounds.

The reaction described is suitable for the estimation of diaminopimelic acid in pure solution; measurements can be made at either 345 or 440 m μ . This technique has several advantages over other colorimetric methods of estimation of amino acids with ninhydrin (Moore & Stein, 1954; Cocking & Yemm, 1954), notably in increased specificity and absence of interference by ammonia. The only common naturally occurring amino acids liable to interfere seriously with the estimation in biological materials are lysine, cystine, tyrosine, tryptophan, proline and ornithine. By choosing suitable reaction conditions, interference can be greatly reduced, but not completely eliminated.

SUMMARY

1. At pH 1 or less ninhydrin reacts with straight-chain α -diaminodicarboxylic acids, including sulphur-containing compounds, and with dibasic amino acids.

2. The spectra of the reaction products, except that with diaminoadipic acid, all show absorption peaks at 320-350 m μ and sometimes also at 430-460 m μ . Diaminoadipic acid resembles ornithine in having its main peak at 515 m μ .

3. Change in acidity affects the shape of the absorption spectrum of diaminopimelic acid only. The absorption at 440 $m\mu$ increases on decrease of pH from 1.0 to 0.3.

4. The reaction can be used for the estimation of diaminopimelic acid, provided that tryptophan, cystine, ornithine, proline and lysine are not present in excess.

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Liver and Brain Mitochondria

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Biochemical studies upon mitochondria may be undertaken from two points of view. The mechanism of the reactions mediated by the catalysts in the mitochondria may be the main interest, or studies may be made of biochemical properties of mitochondria in order to gain information to be applied to the physiology and biochemistry of the whole animal. Such points of view may overlap, but essentially the approach to the preparation and the environment in which the mitochondria are to be studied is different. For the study of mitochondria conditions may be used in which the reactions occur most efficiently; for correlation with studies of the whole animal the mitochondria should be undamaged and in a medium that resembles their normal intracellular environment. We are at present interested in the toxic action of 2:4-dinitrophenol and trialkyltin compounds, both of which act on the processes of oxidative phosphorylation in mitochondria (Loomis & Lipmann, 1948; Aldridge & Cremer, 1955). Such processes are particularly sensitive to modifications in the structure of mitochondria during their isolation and in their subsequent examination. If biochemical studies on these toxic agents are to be correlated with the physiology and pathology of the intoxicated animal, it seems essential to use mitochondria under natural conditions.

From a preliminary survey of the media which have been used for isolation and suspension, it was clear that they varied considerably in their tonicity and composition. Recent work (Opie, 1949; Opie & Rothband, 1953; Robinson, 1950, 1952) has cast doubt on the accepted concept that the cells of the

tissues are isotonic with blood (Peters, 1944). However, an isotonic solution to maintain the cell at its normal volume must contain substances which do not penetrate the cell (Davson, 1951). Since aqueous sodium chloride was used by these workers, and Leaf (1955, 1956) has shown that when tissues swell in Krebs-Ringer solutions sodium chloride passes into the cells, there is no evidence to support the concept that the contents of cells are hypertonic with respect to the extracellular fluid. Osmotic equality between intracellular and extracellular fluids agrees with the work of Deyrup (1953), who showed that slices of renal cortex do not swell in solutions of non-penetrating substances such as sucrose, maltose and lactose. The tonicity of the cytoplasm has been taken as 0.3 osmolar (Krebs & Henseleit, 1932; Conway & McCormack, 1953). In the intracellular fluid the cations present are potassium and magnesium and the bulk of the anions are phosphorus compounds (Hastings, 1940; Manery, 1954). The concentration of the main constituents of the intracellular fluid of the liver and brain (Table 1) have been calculated on the basis that chloride ions are extracellular (Truax, 1939; Hastings, 1940; Lowry, 1943; Manery, 1954) and the assumption that the other electrolytes are all in solution in the intracellular water. The results of these calculations for liver and brain are similar and a medium with a tonicity of 0.3 osmolar for manometric experiments has been prepared with the proportions of potassium:magnesium:inorganic + nucleotide + phosphocreatine phosphorus, the same as those in the calculated composition of the intracellular fluid.