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The Isolation of $a\epsilon$-Diaminopimelic Acid from *Corynebacterium diphtheriae* and *Mycobacterium tuberculosis*

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Hydrolysates of *Corynebacterium diphtheriae* have been shown by paper chromatography to contain an unknown neutral α-amino-acid (Work, 1949*a*, 1950*a*). Identical spots on chromatograms were also found by Klungsøyr & Synge (footnote, Work, 1950*b*) using acid hydrolysates of rumen contents of sheep, and by Asselineau, Choucroun & Lederer (1950) using an acid hydrolysate of an antigenic lipopolysaccharide from *Mycobacterium tuberculosis*. Preliminary reports have already been published on the isolation and identification of this amino-acid from *C. diphtheriae* (Work, 1949*b*, 1950*b*); the present paper gives details of its isolation, degradation and identification.

The behaviour of the unknown amino-acid on paper chromatograms has already been fully described ('neutral substance', Work, 1950*a*). Fig. 1 shows its position in relation to the commonly occurring amino-acids on the phenol-collidine two-dimensional chromatogram. The substance gives a spot which is practically identical in position with those given by ethanolamine-*O*-phosphoric acid and cystine. It can be distinguished from cystine by its stability to hydrogen peroxide, and from ethanolaminephosphoric acid by its behaviour during electrodialysis. Since there was no distinctive chemical or biochemical test by which the amino-acid could be recognized, its isolation was only possible through constant use of paper chromatography, each step being followed on two-dimensional chromatograms. The method finally used for the isolation from hydrolysates of *C. diphtheriae* depended on properties already reported (Work, 1950*a*), namely retention with the acidic amino-acids on acid-treated alumina and failure to migrate in an electric field. Acid-treated alumina was shown by Wieland (1942) to act as an anionic exchanger for acidic amino-acids; accordingly, in early attempts to purify the amino-acid, removal of the acidic constituents from the bacterial hydrolysate was investigated using columns of acid-treated alumina. When the amount of acid-treated alumina relative to the total acidic amino-acids of the hydrolysate was roughly of the order used by Wieland, only the acidic amino-acids were retained on the column; but when the proportion of alumina was increased three times, the unknown amino-acid also was retained, while the bases and the other neutral

components of the hydrolysate were washed through. Ammonia eluates from the alumina columns then contained the unknown amino-acid mixed only with acidic substances, and the unknown neutral amino-acid was thus freed from other neutral substances. Separation from the acidic substances in the ammonia eluate was carried out by electrodialysis, the unknown amino-acid remaining immobile while the acidic substances migrated towards the anode. The immobile neutral aqueous fraction was concentrated without further purification and the unknown amino-acid crystallized.

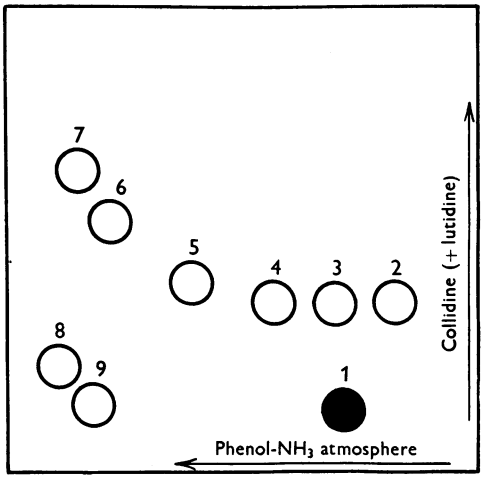


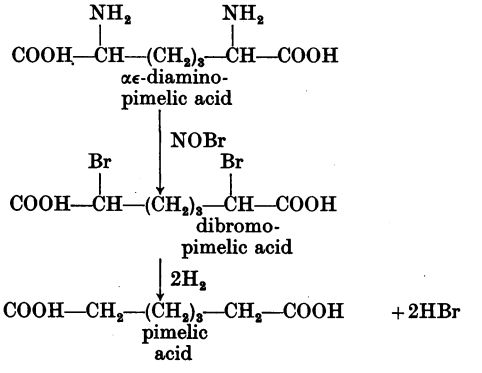
Fig. 1. Position of diaminopimelic acid on phenol-NH_3/collidine-lutidine chromatograms. 1, diaminopimelic acid; 2, aspartic acid; 3, glutamic acid; 4, glycine; 5, alanine; 6, leucine; 7, valine; 8, arginine; 9, lysine.

Identification. The crystalline product, in the form of white needles (Fig. 2), did not melt below 305°. It was soluble in hot water and dilute acids and alkalis, moderately soluble in cold water and insoluble in ethanol and all organic solvents. Elementary analysis gave an empirical formula $C_7H_{14}N_2O_4$, and Van Slyke ninhydrin analysis showed that all the nitrogen was α-amino nitrogen. Although resembling the neutral amino-acids in its behaviour during electrodialysis, the compound exhibited some similarity to cystine and to the acidic amino-acids in its position on paper chromatograms and its retention on acid-treated alumina; it also formed a barium salt insoluble in 50% ethanol. These facts suggested that the substance might be an αα′-diamino-dicarboxylic acid.

All the straight-chain αα′-diamino-dicarboxylic acids $COOH.CH(NH_2).(CH_2)n.CH(NH_2).COOH$ up to diaminosebacic acid ($n = 6$) have been synthesized (Neuberg & Neumann, 1905; Neuberg & Federer, 1906; Sørensen & Andersen, 1908; Carter, van Abeele & Rothrock, 1949), but they have not yet been found to occur in nature. With the exception of diaminopimelic acid ($n = 3$), these amino-acids are reported to be insoluble in cold water. The properties of diaminopimelic acid (Sørensen & Andersen, 1908) resembled closely those of the unknown substance; this fact, coupled with the elementary analysis, gave reason to assume that the unknown amino-acid might in fact be αε-diaminopimelic acid. Comparison of the naturally occurring amino-acid with synthetic specimens of αγ-diaminoglutaric, αδ-diaminoadipic and αε-diaminopimelic acids strengthened this assumption. Diaminopimelic acid resembled the unknown amino-acid in solubility, crystalline form and chromatographic behaviour, 5 μg. giving a purple spot on a chromatogram indistinguishable from that of the natural material. Diaminoadipic acid was insoluble in water and could not be detected on chromatograms, while diaminoglutaric acid was slightly soluble in water and gave a typical purple colour with ninhydrin, but 50 μg. on a paper chromatogram gave only a weak brown spot well away from that given by the unknown amino-acid (see Work, 1950*a*).

The new amino-acid was converted to the benzoyl derivative, the properties of which were compared with those of authentic αε-dibenzamidopimelic acid. Both compounds were soluble in aqueous ethanol and ethyl acetate. This solubility of both dibenzamidopimelic acid and the free amino-acid were contrasted by Sørensen & Andersen (1908) with the general insolubility of diaminoadipic acid and its dibenzoyl derivative. The benzoylated amino-acid from *C. diphtheriae*, although showing slightly different crystalline form and melting point from those of the synthetic derivative, produced no depression of melting point of the synthetic compound and analysed correctly for dibenzamidopimelic acid. The differences between the crystals of the synthetic and natural derivatives could be accounted for by differences in optical form, since the molecule possesses two asymmetric carbon atoms.

Degradation. Confirmation of the assumption that the natural product was indeed diaminopimelic



acid was obtained by degradation. The amino-acid was brominated with nitrosyl bromide and reduced catalytically; the product was identified as pimelic acid by its melting point and by the fact that it did not depress the melting point of an authentic specimen of pimelic acid. As the Van Slyke analysis had already demonstrated that all the nitrogen of the molecule was in the form of α-amino nitrogen, the only possible structure for the compound is αε-diaminopimelic acid.

Optical form. Diaminopimelic acid isolated from *C. diphtheriae* was found to be optically inactive. The compound is probably the internally compensated meso-isomer in which one α-carbon atom has the configuration of D-alanine and the other α-carbon atom that of L-alanine, since the method of preparation was such as to render unlikely the alternative explanation of optical inactivity, i.e. racemization. Four separate batches of material were examined for optical activity, in one of which ammonia was removed by ion exchange instead of vacuum distillation; the possibility seems remote that racemization should have occurred in all four preparations. However, proof of optical form must await resolution of synthetic material.

Dr K. Burton has examined the behaviour of natural diaminopimelic acid towards the L-amino-acid oxidase of *Neurospora crassa* (see Bender & Krebs, 1950) and reports as follows: 'αε-Diaminopimelic acid is readily oxidized by the L-amino-acid oxidase of *N. crassa* with the consumption of one atom of oxygen and the liberation of one molecule of ammonia/molecule of amino-acid added. The rate of oxidation is about the same as that of L-phenylalanine. The quantities of oxygen absorbed and of ammonia produced correspond to the oxidation of half the amino groups to keto groups. This result could be explained by the assumption that the diamino-acid was a racemate. However, the ninhydrin colour reaction became negative, indicating that both α-amino groups had disappeared. No 2:4-dinitrophenylhydrazone was detectable on addition of the hydrazine, indicating that no ketone was formed. A possible explanation of these results is the assumption that α-keto-ε-aminopimelic acid is primarily formed and undergoes secondary ring enclosure to give a tetrahydropyridine derivative (cf. Gabriel, 1908; Lipp, 1896).' Dr E. Bonetti (private communication) found that natural diaminopimelic acid was not oxidized by either ophio L-amino-acid oxidase or kidney D-amino-acid oxidase.

Natural distribution. Since Klungsøyr & Synge and Asselineau *et al.* (1950) also found spots matching diaminopimelic acid in chromatograms from other substances of bacterial origin, it was of interest to examine the distribution of the new amino-acid among micro-organisms. Work on this aspect is in progress and will be fully reported elsewhere, but it can be said here that, to date, the amino-acid has been identified in chromatograms from some twelve bacterial species including *M. tuberculosis* (human, bovine, B.C.G. and avian strains), but has not been found in all bacteria examined, neither has it been found in any product of non-bacterial origin. Crystalline diaminopimelic acid was also isolated from *M. tuberculosis* (human strain), thus confirming the chromatographic identification of the amino-acid in this organism.

The concentration of diaminopimelic acid in different bacteria is variable, but it appears that the amino-acid is by no means a minor constituent. In ethanol-extracted *C. diphtheriae*, for example, diaminopimelic acid accounts for about 2% of the insoluble cell nitrogen, and thus equals the concentration of many other amino-acids in this organism; a small amount was also found with the extractable amino-acids in the ethanolic extract (Work, 1949*a*, 1950*a*). The amino-acid was found neither in tuberculin (see also Asselineau *et al.* 1950), nor in diphtheria toxin, both exogenous proteins produced by organisms known themselves to contain diaminopimelic acid.

EXPERIMENTAL

Isolation of diaminopimelic acid from Corynebacterium diphtheriae

Material. Dried *C. diphtheriae* (P.W. 8 strain) (extracted with 65% (v/v) ethanol, Work, 1949*a*, or unextracted) was hydrolysed for 24 hr. under reflux with 6N-HCl (800 ml./100 g. dry wt.). The hydrolysate was evaporated to dryness *in vacuo*, redissolved in water and dried *in vacuo*; this was repeated three times, but before the last evaporation, humin was removed by filtration and washing with hot water. The residue was dissolved in water (3 ml./1 g. of dried cells).

Alumina columns. Alumina (Savory & Moore, for adsorption purposes) was converted to the acid form by stirring for 15 min. with N-HCl (3 l./kg. Al_2O_3) and washing by percolation with water overnight until the washings had pH 5–6 (Wieland, 1942). Four columns, 4·5 cm. in width and 60–80 cm. long, were packed with acid-treated Al_2O_3 (1 or 1·5 kg. according to length) in water, and were allowed to settle over 24 hr. During all subsequent operations, percolation through the columns was speeded up by maintaining a nitrogen pressure equivalent to 15 cm. Hg above the Al_2O_3. After removing the supernatant water from the columns, neutralized bacterial hydrolysate (50 ml. on the 1·5 kg. columns and 33 ml. on the 1 kg. columns, Work, 1950*a*) was run into the columns, which were washed with water (7–8 l.) until the percolate was substantially free from amino-acids (no ninhydrin colour produced by 3 drops dried successively on filter paper). The percolate was discarded and the columns were eluted with N-NH_3 solution.

Each column before elution had a brown band 2–3 cm. in width at the top, a narrow pink band about 10 cm. down, with sometimes a narrow yellow band between the two; the rest of the column was white. The pink band moved just behind the NH_3 front, and collection of eluate was started

when this band reached the bottom of the column and the percolate became pink and alkaline. The percolate just before the pink band contained the bulk of the Cl^- which had remained on the column, but Cl^- could not be completely eliminated from the eluate owing to the fact that the amino-acids commenced to come off with the pink band, when some Cl^- was still present. It was not possible to separate diaminopimelic acid from the acidic amino-acids as all came off the column together throughout the elution. Elution was continued until 3 drops of percolate on filter paper no longer gave a positive ninhydrin reaction (7–8 l.).

The eluate was concentrated and freed from NH_3 by vacuum distillation, with bath temperature below 60°. NH_3 was removed by ion exchange prior to concentration from one batch of eluate using Amberlite IRC/50 (Resinous Products and Chemical Co., U.S.A.), buffered at pH 4·7 with M-acetate buffer. This batch was worked up separately.

The columns were regenerated *in situ* by passing N-HCl through until the percolate was acidic and slightly cloudy, after which they were washed with water until the percolate had pH of between 5 and 6 (about 10 l.); re-cycling could be continued indefinitely.

Electrodialysis. A four-compartment cell of 14 cm. diam. Perspex tubing with two inner compartments of width 2·5 cm. and end compartments of width 3·5 cm. was used. Thin platinum wire electrodes (0·01 in. (0·25 mm.) diam. and 3 ft. (91·5 cm.) length) were attached to Perspex end plates, on the opposite side of which cooling water was passed. The centre compartments were not cooled, as the main heating occurred at the anode and cathode. Formalized parchment (Campbell, Work & Mellanby, 1951) was used for the semi-permeable membranes separating the cells, as it allowed passage of the acidic amino-acids to the anode much faster than did cellophan.

Concentrated filtered eluate (equiv. to 70 g. dried cells) was placed in the compartment (subsequently called 'centre') next to the cathode, other compartments were filled with water and a potential difference of 220 V. was applied to the electrodes. The current rose rapidly and was maintained at 1·0 amp. by adjusting the voltage. As chloride migrated to the anode, the contents of which were replaced by water frequently, the current stayed constant and finally fell slowly to about 0·02 amp. The pH of the centre compartment was recorded, but not regulated, since diaminopimelic acid remained immobile under all pH conditions provided there were no basic amino-acids moving to the cathode (see Work, 1950*a*). At the beginning, the pH was about 2·5–3; once all the Cl^- had reached the anode, leaving the compartment next to the anode less acidic, the acidic amino-acids migrated to this compartment, accompanied by most of the colour originally present and the 'centre' compartment reached neutrality. The electrodialysis was continued for several hours after the current had reached the minimum value, the total time being 24–30 hr. Occasionally the pH of the centre compartment rose as high as 10, while the catholyte was less alkaline than usual and the current dropped unusually quickly; a dark coating was found on the cathode electrode and was removed by washing with dilute acid. Because of this periodic coating of the cathode wire, it was rinsed with dilute acid between every batch.

The pale-yellow liquid from the 'centre' compartment was chromatographed on paper to ensure that diaminopimelic acid was the only amino-acid present. If any aspartic or glutamic acid were detected, the solution was electrodialysed for a further period of 5 or 6 hr.

Crystallization. The liquid from the 'centre' compartment was decolorized (charcoal) and concentrated until the hot solution was milky in appearance; on cooling, a large mass of crystals of diaminopimelic acid appeared. To recrystallize the material, it was necessary to dissolve in about four times the volume of water from which it originally crystallized and then concentrate. Crystallization could also be effected by addition of ethanol to a concentrated aqueous solution until a faint cloudiness appeared. Material for analysis was crystallized twice from water and once from aqueous ethanol.

Yield. The average yield from many batches of hydrolysate was 1 g. of crude diaminopimelic acid from 150 g. of dried, extracted *C. diphtheriae* cell residues. This represents 40% of the expected yield, since the hydrolysed ethanol-extracted cells contained approx. 2% of their total N as diaminopimelic acid (estimated by paper chromatography, Work, 1950*a*; Lindan & Work, 1951).

Properties of the natural amino-acid

Diaminopimelic acid crystallized from water as fine white needles (Fig. 2) and its hydrochloride as elongated hexagonal plates; the free amino-acid did not melt below 305°, the hydrochloride decomposed about 240°. The free amino-acid was found to be freely soluble in dilute acids and alkalis but to dissolve only with difficulty in water even on heating; at 21° a saturated solution was found to contain 0·92% (w/v) of the amino-acid. Diaminopimelic acid formed a Ba salt insoluble in 50% (v/v) ethanol: precipitation as the Ba salt was a useful method for recovery from crude mother liquors.

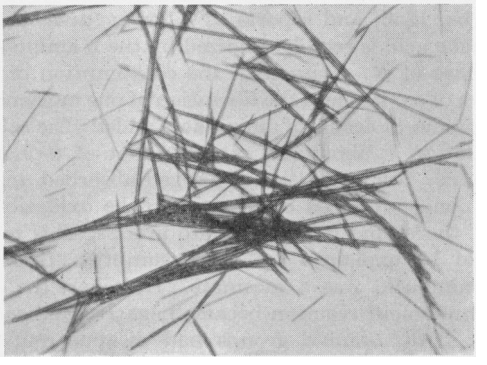


Fig. 2. Crystals of diaminopimelic acid from *C. diphtheriae* (×380).

No optical rotation was observed for diaminopimelic acid in 2·75% (w/v) solutions in 6N-HCl, 12N-HCl or 2N-NaOH (2 dm. tube). Four separate batches prepared at different times were examined, from one of which NH_3 had been removed by ion exchange prior to vacuum distillation.

Identification of the natural amino-acid as αε-diaminopimelic acid

The following analytical figures were obtained: C, 44·3; H, 7·45; N, 14·8. $C_7H_{14}N_2O_4$ requires C, 44·2; H, 7·42; N, 14·8%. α-Amino N, 14·4% (ninhydrin-CO_2 method, pH 2·5; Van Slyke, Dillon, MacFadyen & Hamilton, 1941), i.e. all N present as α-amino N. The free amino-acid was not sufficiently soluble in camphor or water for molecular weight determination.

Dibenzamidopimelic acid. Natural diaminopimelic acid (40 mg.) was dissolved in 1 ml. of 0·5N-NaOH, and three separate additions were made of benzoyl chloride (total 0·28 g.) followed immediately by 2N-NaOH (total 1·8 ml.), with cooling and shaking for about 5 min. after each addition (Sørensen & Andersen, 1908). After standing 10 min. the mixture was filtered, acidified to pH 2–3 with 3N-HCl and kept at 0° for 30 min.; the precipitate was filtered off, washed with water, dried *in vacuo* over H_2SO_4 and thoroughly washed with light petroleum to remove benzoic acid. The crude product weighed 77 mg. (yield 87% of theoretical). Two crystallizations from 25% (v/v) ethanol-water produced a mixture of plates and rosettes; m.p. 181–199° (microstage) unchanged on further recrystallization from the same solvent. Subsequent crystallization from ethyl acetate-ligroin produced fine needles, m.p. 193° (capillary tube). (Found: C, 63·7; H, 5·9; N, 6·9. $C_{21}H_{22}N_2O_6$ requires C, 63·3; H, 5·6; N, 7·0%.) Synthetic dibenzamidopimelic acid from Prof. K. Linderstrøm-Lang also melted at 181–199° on the microstage; recrystallization from 25% ethanol and from ethyl acetate-ligroin produced inhomogeneous crystals, m.p. 195–197° (capillary tube), mixed m.p. with natural dibenzoyl derivative 195°.

Degradation to pimelic acid. Pure natural diaminopimelic acid (107 mg.) was dissolved in 3 ml. 2N-H_2SO_4 containing 392 mg. KBr, and $NaNO_2$ (152 mg.) was added with cooling in small portions over 1·75 hr. (Neuberger & Sanger, 1944). Violent frothing with brown fumes occurred after each addition; after 30 min. the solution turned pale green, and a white solid crystallized out. When additions were completed the mixture stood at 0° for a further 30 min., until most of the bubbling had stopped, and after aeration (N_2) for 30 min., was extracted with ether in a continuous extractor. The dried ethereal extract yielded 150 mg. of oily material which could not be crystallized, but was fractionated by dry ether into two portions. The dry ether-soluble fraction (104 mg.) dissolved in 50% (v/v) ethanol, was hydrogenated in alkaline solution at room temperature and atmospheric pressure with Pt black as catalyst. H_2 uptake was 100% of theoretical for 104 mg. dibromopimelic acid. After acidification, the solution was extracted with ether in a continuous extractor; the dried ethereal extract yielded a waxy solid (50 mg.) which was crystallized three times from dry ether, m.p. 102° (microstage) (pimelic acid, m.p. 104°; mixed m.p. with degradation product 103°), overall yield 55% theoretical.

Identification of diaminopimelic acid in Mycobacterium tuberculosis

Dried steam-killed *M. tuberculosis* of human, B.C.G., bovine and avian strains were each hydrolysed as described for *C. diphtheriae*. Paper chromatograms of the hydrolysates (equiv. to 3 mg. dried cells) showed the diaminopimelic acid spot in all cases. Each hydrolysate was electrodialysed in a small three-compartment cell (capacity of centre 10 ml.) and samples (equiv. to 3 mg. dry cells) of the centre compartment were chromatogramed after treatment with H_2O_2 and ammonium molybdate (Dent, 1948). Diaminopimelic acid spots were found on all chromatograms.

Diaminopimelic acid (Found: C, 43·9; H, 7·3; N, 14·5%) was isolated from hydrolysates of *M. tuberculosis* (human strain), the method of preparation being identical to that used in the case of *C. diphtheriae*.

Examination of tuberculin and diphtheria toxin for diaminopimelic acid

Chromatograms of hydrolysates of tuberculin and diphtheria toxin (untreated and toxoided) showed no diaminopimelic acid spot, even when more than 1 mg. of starting material was used. With this large amount of material, the glutamic acid spots were so large as to obscure any weak diaminopimelic acid spots, so the hydrolysates were electrodialysed and the neutral fraction put on chromatograms in amounts up to 6 mg. of starting material. Diaminopimelic acid was not found.

DISCUSSION

Asselineau *et al.* (1950) reported that diaminopimelic acid was absent from defatted cells of *M. tuberculosis* (human strain) and from tuberculin and present only in the ether-soluble antigenic lipopolysaccharide fraction, which contained in addition only two other amino-acids, alanine and glutamic acid. I have confirmed the absence of diaminopimelic acid from tuberculin, but, having isolated the amino-acid from the human strain of *M. tuberculosis*, suggest that the antigenic lipopolysaccharide (comprising only 7·6% of the total cells) could not be contributing the whole content. A later report by Asselineau & Lederer (1950) showed that diaminopimelic acid is only present in the lipopolysaccharide from virulent strains, being absent from the fraction from B.C.G. strain; since I found the amino-acid to be present in whole cells of B.C.G. strain, it must occur in some cell constituents other than the extractable lipopolysaccharide.

The failure of Asselineau *et al.* to find diaminopimelic acid in chromatograms of *M. tuberculosis* cells may be attributed to the fact that the detection of the amino-acid in chromatograms from crude cell hydrolysates is sometimes difficult because it is directly below the glutamic acid spot, which sometimes may be so large as to obliterate or fuse into the weaker diaminopimelic acid spot. When examining a material for diaminopimelic acid, it is advisable to electrodialyse it, then to examine chromatograms of the neutral fraction. Electrodialysis removes the acidic amino-acids, thus preventing obliteration by glutamic acid; it will also prevent diaminopimelic acid from being confused with ethanolamine-*O*-phosphoric acid, which runs identically on phenol-collidine chromatograms. Confusion with sulphur-containing amino-acids can be prevented by investigating stability of the neutral spot to hydrogen peroxide (Work, 1950*a*).

A figure for the diaminopimelic acid content of ethanol-extracted *C. diphtheriae* has been arrived at, using paper chromatography as a means of estimation. This method is admittedly rough and unless certain precautions are followed (Lindan & Work, 1951) can give very unreliable figures. This accounts for the high value of 4–8% of the total nitrogen

originally reported (Work, 1949*b*); the figure is now calculated to be about 2%.

Diaminopimelic acid is the first diamino-dicarboxylic acid, other than cystine, to be identified in nature. In this connexion it should be pointed out that the presence of its lower homologues diaminoglutaric and diaminoadipic acids would be much harder to demonstrate in view of their insolubility and the probable difficulty of identifying them by paper chromatography. Sørensen & Andersen (1908) report that diaminoadipic acid is soluble only in 5N-HCl, so, although it would be in solution in 6N-HCl hydrolysates, it would precipitate as soon as the HCl was removed. Diaminoglutaric acid, although soluble in mineral acids, does not give a well defined spot on chromatograms. The literature contains little information on the chemistry of $\alpha\alpha'$-diaminodicarboxylic acids, and no explanation has been advanced for the poor solubility in water of all the members of the series except for diaminopimelic acid. Spontaneous ring closure to double lactams or polymerization to polyamides could account for the absence of typical spots on chromatograms of diaminoglutaric and diaminoadipic acids. This lactamization could occur either during preparation or on the paper and would lead to a negative ninhydrin reaction, since such 5- or 6-membered rings would not contain free amino groups. Lactamization could not, however, explain the insolubility of the substances. It is interesting to note that $\beta\gamma$-diaminoadipic acid and its lactam are water-soluble (Traube, 1902). If insolubility were due to polymerization, it is difficult to understand why diaminopimelic acid is not also insoluble; by analogy with the ω-monoamino-monocarboxylic acids, one would expect diaminopimelic acid to polymerize more easily than its two lower homologues, since in the monoamino-acid series, γ-aminobutyric acid and δ-aminovaleric acids lactamize fairly completely on heating, while ϵ-aminocaproic acid (the monoamino-acid corresponding to diaminopimelic acid) tends to polymerize rather than to lactamize to a 7-membered ring (Carothers, 1931). The higher homologues among the diaminodicarboxylic acids are not as insoluble as the lower homologues, being soluble in dilute acids and alkalis.

The low yields of diaminopimelic acid from *C. diphtheriae* might be due to a certain amount of polymerization during preparation. The appearance of a dark humin-like precipitate was often noted, and even during the acid hydrolysis of synthetic dibenzamidopimelic acid to the free amino-acid, a similar precipitate appeared.

If diaminopimelic acid, as isolated from *C. diphtheriae*, proves in fact to be the internally compensated form suggested by its optical inactivity, it contains one carbon atom of the configuration hitherto regarded as unnatural. Since definite confirmation of the optical form has not yet been obtained, speculation of function in relation to optical configuration is premature. The oxidation by *Neurospora* L-amino-acid oxidase with complete disappearance of ninhydrin reaction, although not proving the meso structure, does indicate that the compound is not the racemate, since in this case only part of the starting material would be oxidized, and the remaining unchanged compound in which both α-carbon atoms have the D-configuration would give the ninhydrin reaction. The lack of oxidation by ophio-L-amino-acid oxidase and kidney D-amino-acid oxidase as opposed to the rapid oxidation by *Neurospora* L-amino-acid oxidase is not surprising, since diaminopimelic acid appears to be confined to micro-organisms, and so might be expected to be unattacked by enzymes of animal origin.

The function and metabolic significance of diaminopimelic acid are at present unknown. Since it did not replace pimelic acid for an exacting strain of *C. diphtheriae* (Hale, private communication), it evidently plays no part in biotin synthesis. It occurs in more than trace amounts, so it may not be a metabolite associated only with synthesis of a trace coenzyme; the fact that its concentration in the insoluble portion of bacteria is of the same order as that of many commonly occurring amino-acids suggests that it might be a constituent of bacterial protein. As diaminopimelic acid has been found so far only in products of bacterial origin, it is of particular interest in being one of those few amino-acids confined to one class of living matter. As such, it might provide a basis for a new line of chemotherapeutic attack on bacteria; if its metabolic path is found to be of importance for growth or pathogenicity, drugs might be designed capable of attacking bacteria through this path and such drug would be non-toxic to a host lacking the amino-acid in its make-up.

SUMMARY

1. An unknown amino-acid appearing on paper chromatograms of hydrolysates of *Corynebacterium diphtheriae* was isolated by means of adsorption from crude hydrolysates on excess acid-treated alumina, followed by electrodialysis of the ammonia eluates.

2. The amino-acid was shown by analysis, degradation and comparison with the synthetic product to be $\alpha\epsilon$-diaminopimelic acid.

3. The natural amino-acid had no optical activity and may be the internally compensated meso-isomer.

4. Diaminopimelic acid was also found in hydrolysates of four strains of *Mycobacterium tuberculosis*, but not in tuberculin. It was absent from diphtheria toxin.

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End-Group Assay in some Proteins of the Keratin-Myosin Group

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The fluorodinitrobenzene (FDNB) method for the detection and estimation of amino groups in proteins (Sanger, 1945) has been applied for the most part to proteins of the soluble corpuscular type. The present study extends the technique to the soluble asymmetric proteins tropomyosin, myosin and fibrinogen, which possess a common intramolecular structure similar to that of α-keratin. For reasons which will be discussed later, the results for fibrinogen are still tentative, and most interest centres at present upon tropomyosin and myosin, which are being studied collaterally from a physico-chemical standpoint (Tsao, Bailey & Adair, 1951). The interpretation of the chemical data is indeed very dependent upon other information, the purity of the protein, its particle weight, and especially upon independent evidence for the existence of sub-units. The difficulty of interpretation arises from the fact that tropomyosin and myosin appear to be built of cyclic polypeptide chains, and the small amounts of terminal amino-acids discovered when dinitrophenyl-protein (DNP-protein) is hydrolysed may be due to very tenacious impurities. Sanger's method in fact, if properly applied, is likely to become the most searching of all tests in the determination of protein purity, and its application has so far been too limited to decide the degree of chemical heterogeneity or contamination in other 'pure' proteins.

MATERIALS AND METHODS

Protein preparations

Ox fibrinogen. The fibrinogen was precipitated from fresh citrated ox plasma by the addition of an equal volume of 2M-potassium phosphate buffer, pH 6·6 (Jaques, 1943). It was dissolved and reprecipitated four times, and stored frozen as a solution in 0·25M-phosphate buffer. Fibrin was prepared by diluting with 2 vol. of water and incubating with a trace of purified thrombin for 2 hr. at 25°. The clot was squeezed, dried and ground in ethanol. The amount of non-clottable material was only 5%.

Rabbit myosin. Preparations *SG*1 and *SG*2 (Tables 1 and 3) were the myosin A of Szent-Györgyi (1947) four times precipitated. Preparations *D*1–*D*3 (Table 4) represent a fraction of myosin A precipitating between 40 and 50% saturation with $(NH_4)_2SO_4$ (Dubuisson, 1946, modified by Szent-Györgyi, 1947). Osmotically, this fraction behaves differently from myosin A itself and is thought to be free of actin (Tsao, 1950).

Rabbit tropomyosin. The original method of Bailey (1948) has been only slightly modified. The chief impurity appears to be a colloidal denatured material. Its removal is facilitated by precipitating the tropomyosin from neutral solution with ethanol and drying in ethanol and ether. The protein is then dissolved in water, dialysed against 0·1M-KCl, and the aggregated protein spun down. The ethanol treatment and dialysis are repeated once more, when a smaller amount of impurity is separated, leaving a clear supernatant liquid.