XV. THE CONSTITUTION AND SYNTHESIS OF SPERMIDINE, A NEWLY DISCOVERED BASE ISOLATED FROM ANIMAL TISSUES.

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THE presence of an unidentified base in the mother-liquors after the isolation of spermine phosphate from tissue extracts was originally observed by M. C. Rosenheim and O. Rosenheim during their early work on spermine and has already been reported [Dudley, Rosenheim and Rosenheim, 1924].

This base has been given the name *spermidine* since it not only occurs in association with spermine but also has been found to be structurally related to the latter substance. Its phosphate is much more soluble than that of spermine and crystallises from the 25 % alcoholic mother-liquor, after the removal of spermine phosphate, on increasing the concentration of alcohol to about 50 %.

The material employed in the present investigation was isolated by this procedure during the large scale preparation from ox pancreas of the spermine used in our recently published work on the constitution of the latter base [Dudley and Rosenheim, 1925; Dudley, Rosenheim and Starling, 1926]. The yield of spermidine phosphate is extremely small, being only one-tenth of that of spermine phosphate, namely about 2 g. from 100 kg. pancreas. Only 10 g. of spermidine phosphate were available but, aided by our recently acquired knowledge of the constitution of spermine, we were able to arrive at a probable constitutional formula for the base, and then to prove its correctness by synthesis.

The properties of spermidine are very similar to those of spermine. This is demonstrated in a general way by the fact that it accompanies spermine through all the stages of isolation from tissue extracts by the three methods already described [Dudley, Rosenheim and Rosenheim, 1924]. Like spermine it gives a pyrrole reaction, it appears in the lysine fraction on applying the method of Kossel and Kutscher, its phosphotungstate is insoluble in acetone, it is optically inactive, it does not reduce permanganate in faintly acid solution

and it yields the characteristic semen-like odour when a solution of its chloroaurate is treated with magnesium.

Analyses of its salts gave figures very close to those of the corresponding salts of spermine but indicated that the ratio of carbon to nitrogen in spermidine is lower than in spermine. The molecular weight of the *m*-nitrobenzoyl derivative of the base, considered in conjunction with the analyses, established the molecular formula of spermidine as $C_7H_{19}N_3$. In view of the striking similarity between spermidine and spermine it was considered probable, therefore, that spermidine might be α -(γ' -aminopropylamino)- δ -aminobutane, $NH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$, a formula which is derived from that of spermine, whose structure we have shown to be

by the replacement of one of the terminal γ -aminopropylamino-groups by an atom of hydrogen. The correctness of this conjecture was proved by the following synthesis:

The final compound proved to be in all respects identical with natural spermidine, whose constitution is thus established.

EXPERIMENTAL.

Spermidine phosphate.

An equal volume of alcohol is added to the concentrated mother-liquor from the preparation of spermine phosphate [Dudley, Rosenheim and Rosenheim, 1924].

Spermidine phosphate crystallises from this solution in lustrous plates, usually contaminated with a small amount of spermine phosphate. The easily soluble spermidine phosphate is dissolved in the minimum requisite quantity of cold water and the spermine phosphate, which remains suspended in the solution, is filtered off. To the filtrate, warmed on the water-bath, alcohol is added until a faint permanent turbidity is produced. On allowing the solution to cool spermidine phosphate crystallises. It can be recrystallised from a hot 20 % solution in water. The crystals so obtained are similar in appearance to those of cholesterol and entirely different from the characteristic spermine phosphate.

Heated in a melting-point tube spermidine phosphate begins to shrink at 150° and melts at $207\text{--}9^\circ$ to an opaque fluid which begins to froth at $218\text{--}20^\circ$ without discolouring.

Analysis. 0.2944 g. lost 0.0463 g. H_2O at 108°

Found: H_2O , 15.73 %
 Calc. for $(C_7H_{19}N_3)_2 \cdot 3H_2PO_4 \cdot 6H_2O$: H_2O , 15.61 %
 0.2341 g. (dry) gave 0.1336 g. $Mg_2P_2O_7$
 0.16155 g. (dry) gave $NH_3 = 16.72$ cc. $N/10 H_2SO_4$ (Kjeldahl)
 Found: P, 15.89 %; N, 14.49 %
 Calc. for $(C_7H_{19}N_3)_2 \cdot 3H_2PO_4$: P, 15.92 %; N, 14.38 %

Spermidine picrate.

This salt was prepared by adding excess of sodium picrate solution to a solution of spermidine phosphate. The picrate, which is sparingly soluble in water, when recrystallised from this solvent is obtained as lemon-yellow needles. Under the microscope these are seen to be composed of long thin laminated plates. Under certain conditions of concentration and rate of cooling the crystals formed are broader, being composed of the same thin laminae fused together and appearing to the naked eye as thin plates. Spermidine picrate melts at $210-12^\circ$ to an oily liquid which almost immediately decomposes and froths slowly up the tube.

Analysis. 0.0473 g. (dried at 102°) gave 0.0924 g. nitron picrate

Found: picric acid, 82.68 %
 Calc. for $C_7H_{19}N_3 \cdot 3C_6H_3O_7N_3$: picric acid, 82.58 %

Spermidine hydrochloride.

3.55 g. spermidine picrate were treated on the water-bath with a mixture of 45 cc. absolute alcohol, 5 cc. concentrated hydrochloric acid and 2.5 cc. water. The picrate dissolved in the hot solution and the hydrochloride crystallised in needles on cooling. The crystals were redissolved by heating and 25 cc. acetone were added. The hydrochloride crystallised from the cooled solution and was filtered off, washed with acetone and dried in a vacuum desiccator: yield 1.04 g. On recrystallisation from a mixture of 50 cc. absolute alcohol and 8.5 cc. concentrated hydrochloric acid it was obtained in glistening thin plates, containing no water of crystallisation.

Spermidine hydrochloride is only very slightly hygroscopic; it is quite stable in a relatively dry atmosphere but slowly deliquesces when the humidity is high.

Analysis. Dried at 100° . 0.1396 g. gave 0.1717 g. CO_2 and 0.1055 g. H_2O
 " 0.1331 g. gave $NH_3 = 15.75$ cc. $N/10 H_2SO_4$ (Kjeldahl)
 " 0.1258 g. gave 0.2121 g. AgCl

Found: C, 33.54 %; H, 8.46 %; N, 16.53 %; Cl, 41.71 %
 Calc. for $C_7H_{19}N_3 \cdot 3HCl$: C, 33.01 %; H, 8.64 %; N, 16.50 %; Cl, 41.85 %

* Slightly high owing to incomplete reduction of oxides of nitrogen during the combustion.

Spermidine chloroaurate.

0.1 g. spermidine hydrochloride was dissolved in 7 cc. water. On addition of 5 cc. 10 % "gold chloride" solution an immediate crystalline precipitate appeared. After standing at 2° for 2 hours the chloroaurate was filtered off and recrystallised from 6 cc. 1 % hydrochloric acid.

It forms thin, lustrous, golden yellow plates; m.p. 220–2° with decomposition.

When a more dilute solution is allowed to stand for some time at 2° the salt crystallises in long thin needles with the same melting-point as the plates.

Analysis. 0.1833 g. dried at 100°, gave 0.0930 g. Au.

Found:	Au, 50.73 %
Calc. for $C_7H_{16}N_3 \cdot 3HAuCl_4$:	Au, 50.72 %

m-Nitrobenzoylspermidine.

To 0.15 g. spermidine hydrochloride, dissolved in 6 cc. *N* NaOH, 0.9 cc. molten *m*-nitrobenzoyl chloride was added and the mixture was vigorously stirred in a small beaker. As soon as the reaction of the liquid became nearly neutral 2 cc. *N* NaOH were added and this addition was repeated as necessary. A sticky white mass was formed which slowly became more solid. When the substance became plastic it was pressed out to a thin cake, the mother-liquor was poured off and 2 cc. *N* NaOH were added. After standing overnight the crude *m*-nitrobenzoyl derivative had become friable and was broken up to a powder, filtered off and washed with water: yield (air-dried) 0.336 g.

It was suspended in 20 cc. absolute alcohol. When warmed on the water-bath the solid melted to an oil which then slowly dissolved. To the filtered solution 20 cc. water were added, the turbid solution was warmed until clear and set aside for a day. The *m*-nitrobenzoyl derivative had then crystallised in aggregates of thick needles. These were filtered off and washed with 50 % alcohol: yield (air-dried) 0.298 g. The substance melted at 102° to a turbid liquid which slowly frothed up the tube without charring at higher temperatures. On recrystallising under the same conditions it behaved in the same manner and the melting-point was unchanged. It was then dried in a vacuum desiccator over H_2SO_4 . On warming the substance (0.28 g.) with 25 cc. absolute alcohol the oil which formed at first rapidly changed to a white solid which went very slowly into solution. From this solution, after standing overnight, the substance crystallised in balls of radiating needles. These were collected and again recrystallised from 20 cc. absolute alcohol. On heating the substance with alcohol it no longer formed an oil but remained solid and dissolved very slowly. 0.2155 g. was obtained which melted at 148–50°. In all probability the low-melting product originally obtained was a hydrate.

Molecular weight determination (Rast).

0.0042 g. was dissolved in 0.0378 g. camphor. Δ 7.5°, m.w. 592.

Calc. for $C_7H_{16}N_3$ (CO.C₆H₄.NO₂)₃: m.w. 592.

Analysis. 0.1590 g. (dried for 2 days *in vacuo* over H_2SO_4) gave 20.8 cc. N₂ at 20° and 728 mm. (Dumas).

Found:	N, 14.37 %
Calc. for $C_7H_{16}N_3$ (CO.C ₆ H ₄ .NO ₂) ₃ :	N, 14.20 %

Spermidine forms a chloroplatinate and a mercurichloride which are very soluble. It also yields a crystalline phenylisocyanate derivative, but these compounds are not suitable for purposes of identification.

SYNTHESIS OF SPERMIDINE.

 α -(γ' -Phenoxypropylamino)- δ -aminobutane hydrobromide.

This substance was isolated as a by-product from the preparation of α : δ -bis(γ' -phenoxypropylamino)-butane hydrobromide used in the synthesis of spermine [Dudley, Rosenheim and Starling, 1926]. The mother-liquor from the recrystallisation of the latter substance was concentrated and, after adding alcohol, was poured into 10 volumes of ether. The white crystalline precipitate (5.8 g.) was recrystallised from 96 % alcohol, separating in thin rectangular plates, M.P. 271–3°.

Analysis. 0.1950 g. gave 0.1896 g. AgBr.

0.3648 g. gave $\text{NH}_3 = 19.6$ cc. $\text{N}/10 \text{ H}_2\text{SO}_4$ (Kjeldahl)

Found: Br, 41.38 %; N, 7.52 %

Calc. for $\text{C}_{13}\text{H}_{24}\text{ON}_2\text{Br}_2$: Br, 41.67 %; N, 7.30 %

 α -(γ' -Bromopropylamino)- δ -aminobutane hydrobromide.

4.6 g. of the above phenoxy-compound were heated in a sealed tube at 100° for 12 hours with 25 cc. HBr solution (Sp. G. 1.7). On shaking the clear solution with ether to remove phenol a crystalline substance was precipitated; this was filtered off and washed with alcohol and ether. After three recrystallisations from absolute alcohol 2.4 g. of prismatic needles were obtained: M.P. 234–5°, which proved to be the required substance.

Analysis. 0.0664 g. gave 0.1014 g. AgBr.

Found: Br, 64.97 %

Calc. for $\text{C}_7\text{H}_{19}\text{N}_2\text{Br}_3$: Br, 64.69 %

 α -(γ' -Aminopropylamino)- δ -aminobutane picrate (synthetic spermidine picrate).

1.5 g. of the above bromo-compound were heated in a sealed tube at 100° for 4½ hours with 19.4 g. of an alcoholic solution of ammonia (16 % by weight). The reaction mixture was then evaporated *in vacuo*. The white solid remaining was dissolved in water, potassium hydroxide was added to give a concentration of about 50 % and the solution was then steam-distilled until the distillate gave only a faint cloud on adding picric acid solution. The first fraction of the distillate (200 cc.) was boiled for 5 minutes to expel ammonia. The whole distillate was then neutralised with hydrochloric acid and evaporated to small bulk. Addition of sodium picrate solution precipitated a picrate which, after recrystallisation, was found to be identical with the picrate of natural spermidine. From this picrate the *hydrochloride*, *chloroaurate* and *m-nitrobenzoyl derivative* were prepared. These compounds displayed exactly the same properties as those of natural spermidine. Mixed samples of the corresponding derivatives of the synthetic and natural bases showed no depression of melting points.

Analyses of synthetic spermidine salts.

0.0448 g. picrate gave 0.0872 g. nitron picrate

Found: picric acid, 82.38 %

Calc. for $\text{C}_7\text{H}_{19}\text{N}_3 \cdot 3\text{C}_6\text{H}_3\text{O}_7\text{N}_3$: picric acid, 82.58 %

0.1558 g. chloroaurate gave 0.0784 g. Au.

Found: Au, 50.32 %

Calc. for $\text{C}_7\text{H}_{19}\text{N}_3 \cdot 3\text{HAuCl}_4$: Au, 50.72 %

As the problem of identifying minute amounts of spermidine may arise in the future a few remarks in this connection may not be out of place. In extracts of animal tissues the base will usually be found in association with spermine. The only feasible way of separating these two substances is to convert them into phosphates and then to remove the very insoluble spermine salt. It is essential to separate the two bases completely in this way before preparing the picrates, since it appears to be impossible to separate the two picrates by recrystallisation. There is a characteristic difference between the melting-points of the picrates: that of spermidine forms an oil at $210-12^{\circ}$ which decomposes almost immediately, whilst that of spermine gradually darkens until it becomes black, softening and decomposing at $248-50^{\circ}$ without first melting. The *m*-nitrobenzoyl derivative of spermidine is valuable for identification of the base since a molecular weight determination by Rast's method [1923] requires no more material than is used in an ordinary melting-point determination and since there is a considerable difference between the molecular weights of the corresponding derivatives of spermine and spermidine. The preparation and analysis of a chloroaurate is not decisive on account of the very slight differences in melting-point and gold content of these salts of the two bases.

It was pointed out in our communication on the structure of spermine [Dudley, Rosenheim and Starling, 1926] that this base is the first example of a naturally occurring compound of the type $\text{NH}_2\cdot\text{R}\cdot\text{NH}\cdot\text{R}'\cdot\text{NH}\cdot\text{R}\cdot\text{NH}_2$ and at the same time the first derivative of trimethylenediamine definitely identified among natural products. It is interesting to find the trimethylenediamino-group in a second naturally occurring base, spermidine, which is the first example found in nature of the closely related type

It seems reasonable to suspect that trimethylenediamine itself may be detected among the bases extracted from fresh tissues; in this connection the fact may be recalled that tetramethylenediamine has already been definitely identified in the mother-liquors after the isolation of spermine from yeast [Dudley, Rosenheim and Rosenheim, 1924].

It is hardly necessary to state that the chemical procedures incidental to the isolation of spermine and spermidine cannot have produced artificially the latter from the former. The two bases undoubtedly exist together in animal tissues, and their close chemical relationship strongly suggests that they may be metabolically related.

SUMMARY.

1. A hitherto undescribed base, having the molecular formula $\text{C}_7\text{H}_{19}\text{N}_3$, has been isolated from extracts of animal tissues.
2. In occurrence and constitution it is allied to spermine and has therefore been given the name *spermidine*.

3. The structural formula of spermidine, proved by synthesis, is

i.e. α -(γ' -aminopropylamino)- δ -aminobutane.

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