THE ALLEGED FORMATION OF ADRENINE FROM TYROSINE.

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A paper by Halle[1] appeared in 1906 in which he brought forward some evidence for the formation of adrenine from tyrosine. He prefaces his experimental results with some theoretical considerations as to the possibility of such formation. The requisite changes consist of four steps.

1. The introduction of a hydroxyl group into the benzene ring, for which he cites among other examples the formation of homogentisic acid from tyrosine in alcaptonuria.

2. The elimination of CO_2 from an amino-acid to form an amine, for example, the production of phenylethylamine from phenylalanine, and the formation of parahydroxyphenylethylamine from tyrosine.

3. The methylation of nitrogen. An analogous change is met with in the formation of methyl pyridylammonium hydroxide from pyridine.

4. The introduction of a hydroxyl group into an aliphatic chain. As an example he cites the formation of β-oxybutyric acid from the higher fatty acids.

Without criticising these theoretical speculations in detail we may point out that the fourth step is precisely the point at which Knoop[2] found that the oxidation in the β position stopped short. Aromatic derivatives of the lower fatty acids were metabolised to benzoic or phenyl acetic acid according as there was an odd or even number of carbon atoms in the side chain.

Halle's experiments consisted in incubating finely minced fresh suprarenal glands with tyrosine under antiseptic conditions. Control experiments without tyrosine were carried out under the same conditions. At the end of six days' incubation at 37° the proteins were

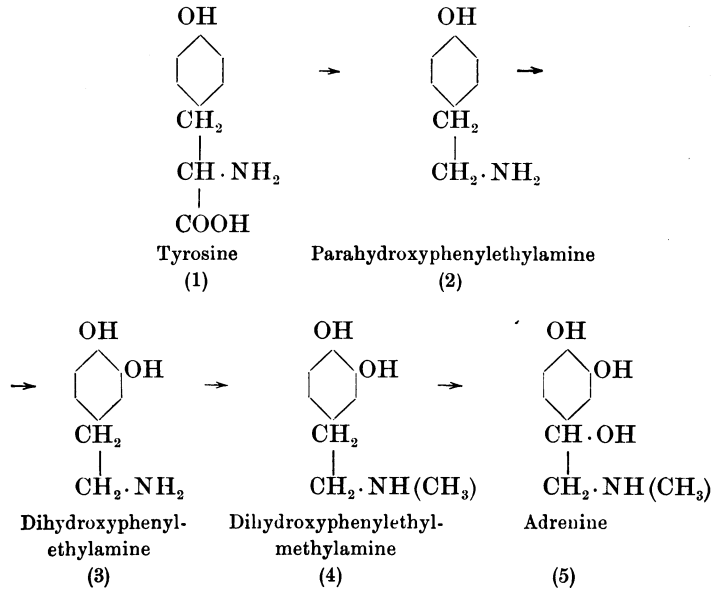
[1] W. L. Halle. *Beitr. z. chem. Physiol. u. Path.* VIII. p. 276. 1906.

[2] *Hofmeister's Beitr.* VI. p. 150. 1905.

removed, the solution concentrated, the adrenine precipitated by ammonia, and the precipitate weighed. In two experiments out of four he obtained results which appeared to indicate the formation of adrenine under these conditions.

The method of estimating adrenine by precipitation with ammonia is worthless. The precipitate obtained under such conditions is, as Halle himself admitted, contaminated with a considerable amount of inorganic matter. The fact that the ash, obtained by ignition of his precipitates, was approximately constant in all cases does not necessarily indicate a constant proportion of impurity. In view of the importance attaching to such a result, if true, it seemed to us desirable to repeat and extend these experiments, employing a method of estimating adrenine which should be capable of more definite interpretation.

The transformation of tyrosine into adrenine, according to Halle's theory, may be represented in the following scheme.



As will be seen later we could obtain no evidence of any of these steps in the case of experiments with tyrosine. We therefore tested the transformation from steps (2) and (4).

The experiment with tyrosine was carried out as follows:

120 grms. of finely minced fresh ox suprarenal glands were placed in each of two flasks. To each flask was added 40 c.c. of saline, 1 c.c.

chloroform and 10 c.c. of toluene. To one flask was also added 0·5 grm. of tyrosine. The mixtures were then incubated for 7 days at 37°. At the end of this time 0·5 grm. of tyrosine was added to the contents of the second flask and the two mixtures worked up separately in exactly the same way, as follows. The mixtures were made faintly acid with dilute acetic acid and heated on a water bath to 80—90°. They were then filtered and the filtrates boiled. The coagulated protein was filtered off and the filtrates neutralised.

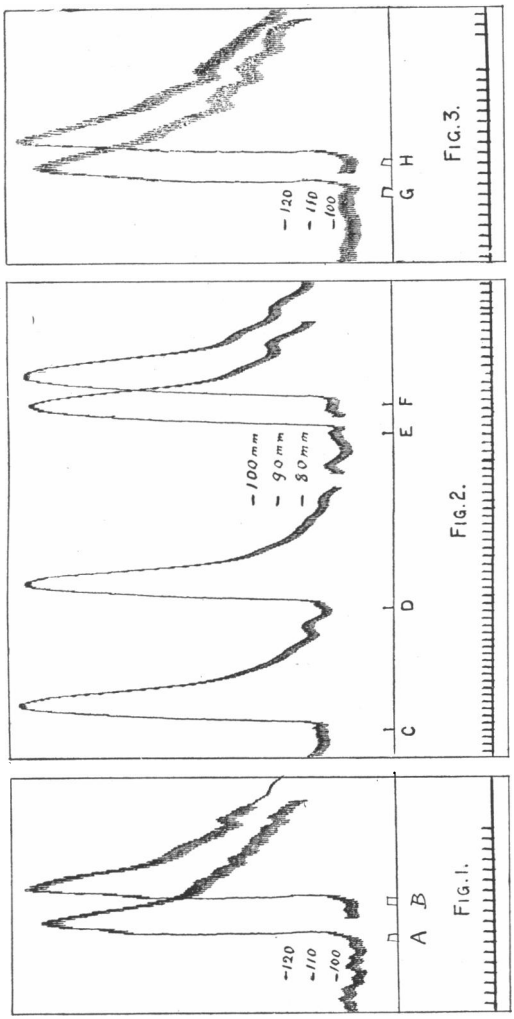


Fig. 1. Decerebrate cat. Artificial respiration, blood-pressure, base line and signal, time in ten seconds. $A = 1$ c.c. of ten times diluted extract of suprarenal glands incubated with tyrosine for seven days. B = control.

Fig. 2. Decerebrate cat. Artificial respiration, blood-pressure, base line and signal, time in ten seconds. C and $E = 1$ c.c. of ten times diluted extract of suprarenal glands incubated with parahydroxyphenylethylamine. D and F = control.

Fig. 3. Decerebrate cat. Artificial respiration, blood-pressure, base line and signal, time in ten seconds. G = control. $H = 1$ c.c. of 20 times diluted extract of suprarenal glands incubated with dihydroxyphenylethylmethylamine.

The adrenine content of the two solutions was compared physiologically. The method employed consisted in determining the rise in arterial blood pressure produced in a pithed cat by intravenous injection of equal submaximal doses of the two filtrates. The rise in blood-pressure was practically the same in each case. (See Fig. 1.)

If one-tenth of tyrosine added had been transformed into adrenine the effect on the blood-pressure would have been doubled. Further, if either dihydroxyphenylethylamine (step 2) or dihydroxyphenylethylmethylamine[1] had been formed the rise of blood-pressure induced by the test filtrate would have been greater than the control. As is readily seen in the figure the control is slightly higher than the test.

We then incubated parahydroxyphenylethylamine with suprarenal glands under similar conditions and similarly controlled. The rises in blood-pressure were here again equal in both cases. (See Fig. 2.) If a twentieth of the added amine had been converted into adrenine the effect on the blood-pressure would have been doubled.

Finally we incubated dihydroxyphenylethylmethylamine[2] under similar conditions. In this case only the last step, the introduction of a hydroxyl group into the aliphatic chain, is required for the formation of adrenine. Comparison of the solution and control showed rises of blood-pressure which were the same within the limits of the experimental error. (See Fig. 3.) If one-seventh of the amine had been converted into adrenine the effect on the blood-pressure would have been doubled.

We are, therefore, of the opinion that there is as yet no evidence of the formation of adrenine by ferment activity, from tyrosine or the more closely related bases parahydroxyphenylethylamine and dihydroxyphenylethylmethylamine.

[1] As yet unpublished work of Dale and G. Barger.

[2] For this base we are indebted to F. L. Pyman. *Trans. Chem. Soc.* xcvii. p. 264. 1910.