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(*From the Wellcome Physiological Research Laboratories, London.*)

That an extract of putrefied horse-meat raises the arterial blood-pressure when injected intravenously, was first shown by Abelous, Ribaut, Soulié and Toujan[1] in 1906. These authors obtained from putrid meat the impure hydrochloride of a chloroform soluble base, which showed a very marked pressor action. Later Dixon and Taylor[2] observed that extracts of human placenta, injected intravenously, similarly caused a rise of blood-pressure, and, in addition, contraction of the pregnant uterus. Rosenheim found, while attempting to isolate the active principle of placenta, that only the more or less putrefied placenta gave active extracts, the fresh or merely autolysed organ failing to do so. He suggested at a meeting of the Physiological Society in January, 1908, that a crystalline hydrochloride obtained by him, was very probably identical with that of the French authors. In order to test this supposed identity, we prepared a quantity of the base from putrid horse-meat and soon found that after complete extraction with chloroform, the aqueous solution still retained a considerable proportion of its original activity[3], due to a second base, insoluble in chloroform, which Abelous and his collaborators had apparently overlooked. This base was only present in small quantities, and the large amount of material necessary for its examination enabled us to identify the chloroform soluble base at the same time. Abelous and Ribaut[4] have more recently analysed an oxalate of this latter base. After we had communicated our results to Rosenheim, he was able to show that one base obtained by him from putrid placenta was identical with that one of our bases from putrid meat which was insoluble in chloroform, and

[1] *C. R. Soc. de Biol.* I. pp. 463, 530. 1906.
[2] *Brit. Med. Journ.* 1907, II, p. 1150.
[3] The physiological experiments were performed for us by Dr H. H. Dale.
[4] *C. R. Soc. de Biol.* I. p. 908. 1908.

that a second chloroform soluble base, which he also obtained, was probably identical with that of Abelous.

Method of putrefaction and preparation of extracts.

In 1906 one of us confirmed Abelous' original observation, working on a small scale with ox hearts; in 1908, however, when we wished to isolate the bases, we found it preferable to use horse-meat and to modify Abelous' method. The muscle was taken from a horse immediately after death, and placed in cold storage. When required, it was freed from fat and tendon, minced, placed in large bottles, each holding 10 kilos. and allowed to putrefy at 37°. The bottles were corked and provided with a tube to conduct offensive gases up the chimney of the incubator room. In Abelous' experiment an equal quantity of water was added, but we omitted this in order to avoid unnecessary evaporation of the extract. The meat was not infected with any particular organism, although we occasionally added a little material from a previous successful putrefaction. The conditions were in some experiments strictly anærobic (carbon dioxide and mercury trap), in others only approximately so; we could not trace any effect on the yield of physiologically active bases. After 6—8 days, when the mass had partly liquefied, the bottles were placed in a steamer and the proteins coagulated at 100° with hydrochloric acid (the only really unpleasant operation). After filtration in the open air the filtrate was evaporated to a thick syrup in a vacuum-pan provided with a sulphuric acid trap, by which means the escape of bad odours into the laboratory was completely obviated. Abelous and his colleagues precipitated the concentrated extract with absolute alcohol, but we found it much better to continue the evaporation further, mix the soft residue with sand and extract with acetone, which is a more powerful protein precipitant than alcohol and yet a sufficiently good solvent for the hydrochlorides of the bases in question. In this way we at once obtained a much cleaner solution and we could omit the precipitation with mercuric chloride employed by the French chemists. After evaporation of the acetone a dark brown liquid remained containing a considerable quantity of fatty acids. This liquid was generally completely miscible with chloroform and its chloroform solution was extracted with dilute hydrochloric acid; in this way the bases were removed, and the fatty acids and colouring matter remained behind in the chloroform layer.

Identification of the base soluble in chloroform.

The acid solution of the bases was washed with chloroform, made alkaline and repeatedly extracted with ether. The ethereal solution was dried with sodium sulphate and precipitated with an ethereal solution of anhydrous oxalic acid. The filtrate from the precipitated oxalates did not contain any pressor substances; the oxalate itself readily crystallised from alcohol and the crystalline oxalate represented nearly the whole of the chloroform-soluble pressor principle; from the mother liquors on concentration a little of a more soluble oxalate eventually separated, but this had no pressor action. After recrystallisation from a mixture of alcohol and acetone the physiologically active oxalate melted at 166° and was analysed. The substance was dried *in vacuo* over sulphuric acid; it was found impossible to dry to constant weight at 100° because of decomposition and loss of base.

0·2080 grm. gave 0·3618 CO_2 and 0·1573 H_2O; C = 47·53, H = 8·40.
0·2046 grm. gave 13·7 c.c. of moist N at 765 mm., and 11° C. = 8·0 per cent. N.

	Found	Calculated for $C_7H_{13}O_4N$
C	47·53	47·46
H	8·40	8·48
N	8·0	7·9

The analyses agree with the formula $C_5H_{13}N \cdot C_2H_2O_4$ which is that of the acid oxalate of an amylamine. By heating the salt with an excess of lime we obtained a small quantity of the free base which boiled at 89—90° and possessed the characteristic smell of a fatty amine. (Isoamylamine is stated to boil at 95°.) We determined its vapour density according to Victor Meyer's method.

0·0745 gave 21 c.c. of moist air at 761 mm. and 10° C. V.D. = 41·5; vapour density calculated for $C_5H_{13}N$ = 43·5.

The free amine was converted into the hydrobromide by adding alcoholic hydrobromic acid to its solution in ether. It formed glistening plates m.p. 225° which were non-deliquescent but extremely soluble in water. The hydrochloride, *i.e.* the salt first obtained by Abelous and his colleagues, was even more soluble and could not be obtained pure.

0·1293 grm. gave 0·1712 CO_2 and 0·0939 H_2O; C = 36·1, H = 8·1.

	Found	Calculated for $C_5H_{13}N$, HBr.
C	36·1	35·7
H	8·1	8·3

The amine was a primary one, for it gave a strong odour of isonitrile on heating with alcoholic potash and chloroform.

Eight isomeric amylamines are theoretically possible, but only two of these, those derived from leucine and from isoleucine, are likely to appear as products of putrefaction. Since the base was optically inactive it could not be derived from isoleucine and was most probably therefore isoamylamine $(CH_3)_2:CH.CH_2.CH_2NH_2$, which is formed from leucine by loss of carbon dioxide.

We therefore compared synthetic isoamylamine acid oxalate with the salt obtained from putrid meat. The former melted at 169°, the latter at 166°, and a mixture of the two at 166°. In the course of our experiments we also obtained from meat an oxalate melting at 200°, which on distillation with lime gave a base which could afterwards be converted into the oxalate of m.p. 166°. This was evidently the normal oxalate of isoamylamine; it melted at 200°, a synthetic specimen melted at 207°, and a mixture of the two salts at 203°. The physiological activity of synthetic isoamylamine was qualitatively and quantitatively identical with that of the base obtained from putrid meat and inferior to that of normal amylamine (see Barger and Dale, *Proc. Physiol. Soc.* Jan. 23, 1909). The observed boiling point of the natural base agreed fairly well with that given for isoamylamine.

We lay stress on the various points proving the identity of isoamylamine with the base from putrid meat; Abelous and Ribaut[1] have assigned to the latter base the provisional formula $C_6H_{11}NO$, based on the analysis of a supposed neutral oxalate melting at 170° and of a platinum salt. Although the melting point given is but slightly higher than that of acid isoamylamine oxalate and much lower than that of the normal oxalate, it is most probable that the French authors were dealing with an impure specimen of the latter salt, which had been partially decomposed by heating at 100°. We have indeed found that the acid salt slowly loses weight at 100°, and this loss of weight is accompanied by a fall in the percentage of carbon, hydrogen and nitrogen, evidently due to a loss of amine. It is therefore intelligible why Abelous and Ribaut found for their (admittedly impure) salt somewhat less carbon, less hydrogen and less nitrogen than is required by the formula $(C_5H_{13}N)_2.C_2H_2O_4$. As most of our material had been prepared by a slight modification of Abelous' process we prepared a quantity of the hydrochloride strictly according to his directions (using alcohol and mercuric chloride), dissolved it in sodium hydroxide and distilled with

[1] *C. R. Soc. de Biol.* I. p. 908. 1908.

steam, when it was found that the whole of the pressor substance had passed into the receiver.

Identification of the second pressor principle.

When the crude acid solution of the bases, after washing with chloroform, was made alkaline and distilled with steam until no more pressor principle passed over, the solution was still strongly active physiologically. The whole of the pressor base in the distillate could readily be extracted by chloroform, but the non-volatile portion could not be extracted at all by this solvent, nor by ether or ethyl acetate. In concentrated aqueous solution it was precipitated by phospho-tungstic acid, but scarcely at all by lead acetate or mercuric chloride. Its isolation became possible by the observation that it was readily removed by amyl alcohol from a solution containing a slight excess of sodium carbonate, but not from a solution containing $\frac{N}{2}$ sodium hydroxide, nor from an acid solution; the substance was thus immediately characterised as a phenolic base. By washing the solution in sodium hydroxide with amyl alcohol, all other bases and the neutral substances were removed; the solution was then neutralised and again made alkaline with a slight excess of sodium carbonate. On extraction with amyl alcohol the pressor principle was now removed in a relatively pure condition. The amyl alcohol was removed by distillation *in vacuo* and steam distillation.

As tyrosine is the only phenolic fission-product of protein, we suspected a relationship of the base to tyrosine, similar to that which we had already observed between the first pressor principle and leucine. In that case the second pressor principle would be *p*-hydroxyphenylethylamine, formed from tyrosine by elimination of carbon dioxide. This hypothesis received support from the fact that the purified aqueous solution of the base gave a strong Millon reaction.

We therefore benzoylated the base according to Schotten-Baumann; after the benzoyl-derivative had been dried on a porous plate, it readily crystallised from alcohol in needles melting at 169°, which were found to be identical with dibenzoyl-*p*-hydroxyphenylethylamine, first prepared by Emerson[1]. On recrystallisation from alcohol the melting point was 170° (uncorr.) and was found to be identical with

[1] *Beitr. z. chem. Physiol. u. Pathol.* I. p. 501. 1902.

that of a synthetic preparation. A mixture of the two also melted at 170°[1].

0·0977 grm. gave 0·2735 CO_2 and 0·0526 H_2O; C = 76·35, H = 6·0.

	Found	Calculated for $C_3H_9ON(C_7H_5O)_2$
C	76·35	76·5
H	6·0	5·5

The aqueous filtrate, after benzoylation, was physiologically inert. The benzoyl derivative was hydrolysed by heating with 20 % hydrochloric acid for two hours to 140°, and was then found to have a strong pressor action. The same physiological effect was observed with specimens of *p*-hydroxyphenylethylamine prepared by sublimation from tyrosine and synthetically by reduction of *p*-hydroxybenzylcyanide. *p*-hydroxyphenylethylamine has a much more powerful pressor action than isoamylamine.

Probable identification of a third pressor principle in putrid meat.

Having thus obtained two pressor substances closely related to amino-acids, we considered whether other amino-acids might not yield pressor bases by a similar elimination of carbon dioxide. Barger and Dale[2] have shown that isobutylamine has a very slight pressor action and it is possible that this substance occurs in putrid meat as a decomposition product of valine; the physiological effect is however too small to lead to the identification of the substance. Phenylethylamine is known to occur in the putrefaction of gelatine, probably as a decomposition product of phenylalanine, and since synthetic phenylethylamine was found to have a powerful pressor action, we attempted to isolate this substance from putrid meat.

For this purpose the amyl alcohol extracts of the $\frac{N}{2}$ sodium-hydroxide solution, obtained in the preparation of *p*-hydroxyphenylethylamine, were used. Steam distillation had been omitted and the quantity used represented 40 kilos. of meat. After removal of the amyl alcohol by distillation, the dark brown residue was dissolved in chloroform, and extracted with hydrochloric acid. After making alkaline, the bases

[1] Dr Rosenheim, having obtained the same benzoyl derivative from putrid placenta, found that it gave Mörner's reaction for tyrosine. At his suggestion we have tested our own substance and find that it also gives this reaction.

[2] *Loc. cit.*

were transferred to ether; the solution was dried and the ether evaporated. On distillation isoamylamine first passed over. A few drops of a fraction boiling between 190° and 210° were collected and dissolved in dry ether. (Phenylethylamine boils at 198°.) On adding alcoholic hydrochloric acid a hydrochloride separated out which was crystallised from alcohol and ether and dried on a porous plate. 2 milligrammes of this produced, on intravenous injection into a pithed cat, the effect illustrated in Fig. 1; the effect is very similar to that produced by the same quantity of synthetic phenylethylamine.

The boiling point of the free base excludes the presence of either of the two pressor substances already described; that of isoamylamine is further excluded by the readiness with which the hydrochloride separated out on adding alcoholic hydrogen chloride to its ethereal solution and by the intensity of its pressor action. Finally, *p*-hydroxyphenylethylamine was absent because the solution did not give the Millon reaction. It is, therefore, very probable that the pressor action of an extract of putrid meat must, to a slight extent, be attributed to the presence of phenylethylamine.

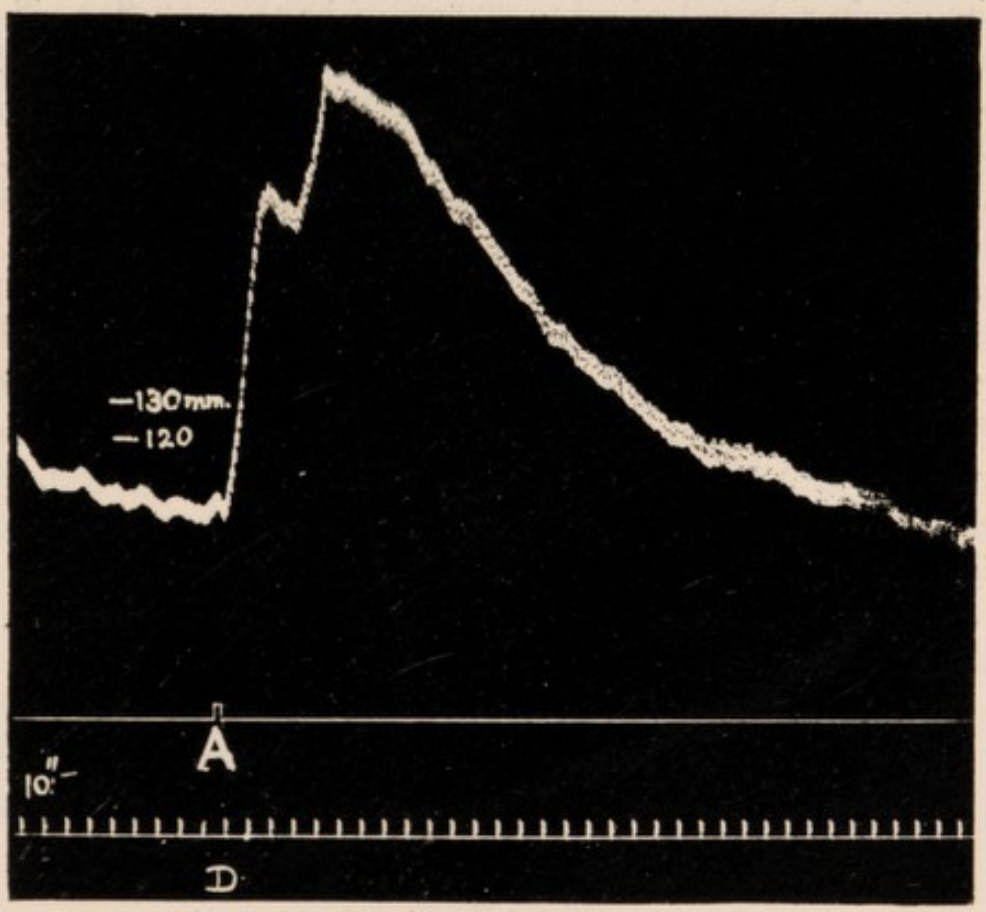


Fig. 1. Carotid blood-pressure of a pithed cat. At *A* 2 milligrams of crystalline hydrochloride (presumably of phenylethylamine) were injected intravenously.

Discussion of results. Production of bases from amino-acids.

The putrefactive formation of isoamylamine has hitherto only been observed in yeast; it has also been stated to occur in cod liver oil prepared by the old method, *i.e.* by putrefaction of the livers, and Nencki[1] obtained an amylamine from cheese. *p*-hydroxyphenylethylamine was found by Emerson[2] in pancreas, autolysed under conditions regarded as excluding putrefaction and the yield was increased by the addition of tyrosine; the same base was found by Langstein[3] in prolonged peptic digestion. Abderhalden[4] was unable to confirm Emerson's results and considers that the *p*-hydroxyphenylethylamine is formed by putrefaction.

It is extremely probable that in each case the base is formed from the corresponding amino-acid, as was proved by Ellinger for putrescine and cadaverine. We were, indeed, unable to obtain an appreciable amount of pressor substance from putrefied broth to which leucine had been added. In the case of tyrosine, however, we obtained a positive result; 1·7 gram of tyrosine was dissolved in the calculated quantity of sodium hydroxide and added to 300 c.c. of broth. After sterilisation the broth was infected with a culture from human fæces, and was allowed to putrefy for 4 days at 37°, together with the same quantity of broth, similarly infected, but without the addition of tyrosine. At the end of the incubation period both samples of broth were precipitated with basic lead acetate; after removal of the lead and concentration, 0·9 gram of tyrosine crystallised out from the one solution and was filtered off. After extraction with amyl alcohol and removal of the solvent by steam distillation, both extracts were examined physiologically. The effect is illustrated by Fig. 2; solution A was prepared from the control broth, solution B from the broth to which tyrosine had been added; in each case 5 c.c. = 150 c.c. of broth were injected. By subsequent injections Dr Dale found that the pressor action of the 5 c.c. of solution B corresponded to 2—3 milligrams of *p*-hydroxyphenylethylamine. As this amount of solution was equivalent to 0·85 gram of tyrosine added to the broth, it follows (on the assumption that the pressor effect observed was indeed wholly due to *p*-hydroxyphenylethylamine) that 0·3—0·5 % of the tyrosine supplied was transformed to *p*-hydroxyphenylethylamine,

[1] *Berichte*, x. p. 1034. 1877.
[2] *Beitr. z. chem. Physiol. u. Pathol.* i. p. 501. 1902.
[3] *Ibid.* i. p. 507. 1902.
[4] *Lehrb. d. physiol. Chem.* Berlin, 2nd edit. p. 353. 1909.

i.e. a quantity of the same order of magnitude as that obtained in the putrefaction of meat, when the amount formed corresponded to something like 0·2 % of the total tyrosine in the meat.

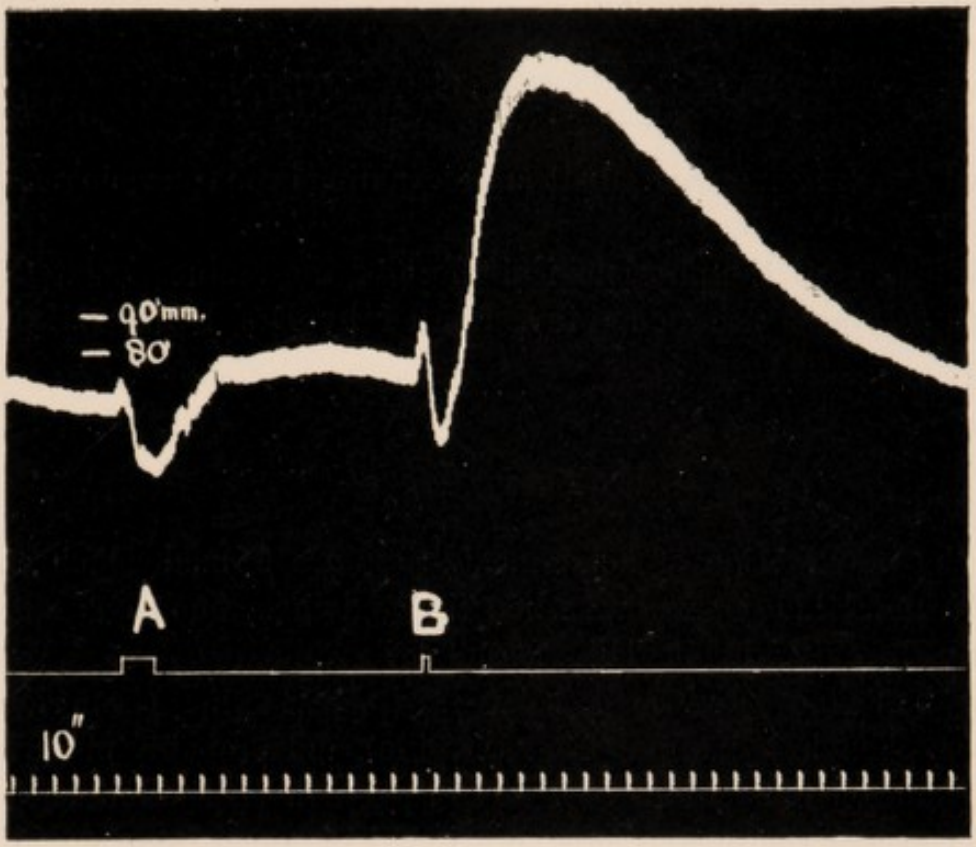
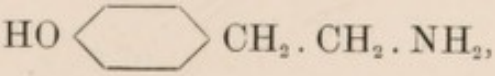


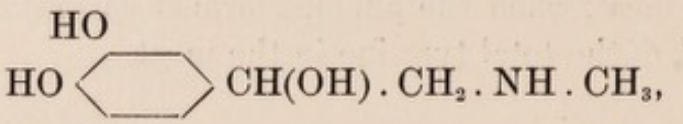
Fig. 2. Carotid blood-pressure of a pithed cat. At *A* 5 c.c. of solution A were injected intravenously, at *B* 5 c.c. of solution B. (See text.)

This result induces us to emphasise the probability that the amines which we have isolated are normally formed by putrefaction in the intestine and are absorbed from it. Abelous indeed suggests that the pressor base he has obtained from urine and named urohypertensine is identical with the substance observed by him in putrid meat (presumably therefore isoamylamine). Although we have evaporated some thirty litres of human urine, we have not been able to obtain enough of the urinary pressor base for identification, and must leave its identity with isoamylamine in doubt. We found, however, that nearly all, if not all, the pressor action is due to a substance which is volatile with steam.

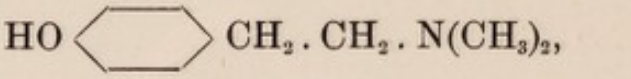
p-hydroxyphenylethylamine,



is related both chemically and pharmacologically to adrenaline



and to the alkaloid hordenine



obtained by Léger[1] from malt and examined pharmacologically by Camus[2].

The details of the physiological effect of the amines isolated by us from putrid meat are being investigated by Drs Dale, Dixon and Taylor.

Summary.

In the putrefaction of horse-meat bases are formed which cause a rise of arterial blood-pressure, when injected intravenously, and which are derived from amino-acids by loss of carbon dioxide.

The following have been isolated: isoamylamine (from leucine), *p*-hydroxyphenylethylamine (from tyrosine), and probably phenylethylamine (from phenylalanine). Of these the second is the most active.

We consider the pressor base, previously obtained from putrid meat by Abelous and his colleagues, to be isoamylamine.

[1] *C. R.* CXLII. p. 108. 1906.

[2] *Arch. int. de Pharmacodyn. et de Thér.* XVI. p. 43. 1906.