THE FATE OF PARAHYDROXYPHENYLETHYLAMINE IN THE ORGANISM. By A. J. EWINS AND P. P. LAIDLAW.

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PARAHYDROXYPHENYLETHYLAMINE was shown by Dale and Dixon[1] to be a physiologically active substance, producing effects of the same kind as those produced by adrenine, although its activity was relatively small. This fact increased the interest of the older observations concerning its wide distribution. The base has been isolated from the products of prolonged pancreas autolysis[2], liver autolysis[3], and peptic digestion of albumin[4]. It has been isolated from cheese[5], from putrid meat[6], and putrid placentæ[7], and recently from liquid extract of ergot[8]. It is formed from tyrosine by bacterial action and it has been suggested that it may be formed by this agency in the alimentary canal. This formation has quite recently been regarded as playing a part in certain pathological states in which a high blood-pressure is the most prominent symptom. Bain[9] has adduced some evidence in favour of its excretion in the urine in such cases. The fate of the base after absorption is therefore of some interest.

With a view to determining the manner in which this substance is metabolised we have carried out experiments of two kinds.

I. Experiments on intact animals.

These consisted in the administration of a suitable dose of the base by the mouth and examining the urine collected in the following 36 hours.

II. Perfusion experiments on isolated organs.

These were performed with a view to determining the part which various organs played in the metabolism of this substance.

I. FEEDING EXPERIMENTS.

These were performed upon dogs. On account of the physiological action of the base we were limited to the administration of comparatively small amounts. We found that 0·5 gm. of the hydrochloride was well borne by an 8 kilo dog. When this amount was given by mouth, in a gelatine capsule, no symptoms were noticed for the first half hour. During the next 10 minutes a series of symptoms developed which may be briefly described as symptoms of sympathetic stimulation. (See also Dale and Dixon, *loc. cit.*) The dog became quiet and stood in one position unless disturbed, thick saliva appeared at the angles of the mouth, and some nausea was noticeable, the hair along the back became erect so that the skin was visible between the hairs, the pupils became maximal, and the heart-beat dropped to 48 per min. and was not quite regular, being obviously under extreme vagus inhibition. Some thick mucoid saliva was vomited. These symptoms persisted for about one hour and then gradually subsided. The urine was collected for 36 hours after the administration of the amine. It was found to give a very much stronger Millon's reaction than normal dog's urine. We were able to show that this Millon reaction was due to an ether-soluble acid. This provided us with a method for isolation.

The urine collected (165 c.c.) was rendered faintly acid with acetic acid and boiled for a few minutes. The solution was then cooled, 10 c.c. of 10 % HCl added, and filtered. The filtrate was shaken out several . times with ether until the ether extracts gave only a faint Millon reaction. The combined ethereal extracts were washed with a small quantity of water, the ether evaporated off and the residue taken to dryness by evaporation with alcohol. The dry product was then extracted with benzene until the extract no longer gave a reaction with Millon's reagent. The benzene solution was taken to dryness, the residue extracted with water, the aqueous solution taken to dryness and the product recrystallised from benzene. In this way we obtained 70 mgms. of an acid which melted at 147—8°. The acid was readily soluble in water, gave a strong, deep red colour-reaction with Millon's reagent, and, when it was mixed with an equal weight of synthetic parahydroxyphenylacetic acid (obtained by the hydrolysis of para-hydroxybenzyl cyanide), the melting point remained unchanged.

The urine, after extraction with ether as above, still gave a very marked reaction with Millon's reagent. Concentrated hydrochloric acid

was added so that the resulting concentration of acid was 5 %, and the urine boiled under a reflux condenser for one hour.

The solution was then again repeatedly extracted with ether. On evaporation an oily residue was obtained which gave a strong Millon reaction but from which nothing crystalline could be obtained. This residue was boiled with concentrated hydrochloric acid for about one hour, cooled, filtered, made alkaline with sodium carbonate and extracted several times with ether. The aqueous solution was then made acid with hydrochloric acid and extracted with ether until the extracts no longer gave a Millon reaction. The ether solution was then worked up for parahydroxyphenylacetic acid, as in the first part of the experiment, when 40 mgms. of acid melting at 146—7° were obtained. The total yield of parahydroxyphenylacetic acid obtained was thus 110 mgms. or 25 % of the theoretical amount possible.

Repetition of this experiment gave similar results, but in no case could we account for more than 25 % of the administered amine. This is doubtless due in part to the great difficulty in isolating a comparatively small amount of the acid from such a complex mixture as urine. It was very probable from the evidence of previous workers that, if parahydroxyphenylethylamine were quantitatively transformed into parahydroxyphenylacetic acid, we would have been able to obtain a larger amount from the urine than we actually did. Thus Schotten[10], taking 7·5 grm. of the acid himself, was able to recover 77·7 % from the urine of the next 24 hours. E. and H. Salkowski[11] administering 2 grms. to dogs recovered 40—50 %. With a view to determining what percentage could be recovered when small doses were given to dogs, we administered 0·5 grm. of the acid (as sodium salt) by mouth, and found 50 % in the urine of the next 36 hours. The amount of oxyphenylacetic acid turned out in the urine in our feeding experiments with parahydroxyphenylethylamine probably, therefore, represents about one half that actually formed during metabolism. In any case it would appear that a considerable part of the administered amine is still unaccounted for. We therefore endeavoured to trace its fate through the organism.

II.   PERFUSION EXPERIMENTS.

These experiments were performed upon isolated organs obtained immediately after death from rabbits and cats. In our earlier experiments we employed a perfusion apparatus modelled on that

used by Locke and Rosenheim[12], when studying the consumption of dextrose by the isolated heart. In our later experiments we employed a more complicated apparatus very similar to that described by Brodie[13]. The form of apparatus used made no difference to the results. Special attention was paid to three points: (1) To start the perfusion as soon as practicable after the death of the animal. (2) Thorough oxygenation of the perfusion fluid. (3) The maintenance of a constant temperature of 37°.

A. *Perfusion of liver.* In the case of liver perfusions the procedure was as follows. The animal was killed, the abdomen opened, the bile duct and hepatic artery ligatured, a cannula inserted into the portal vein, and a little saline washed in to clear the neck of the cannula from blood and thus avoid clotting. (A small tributary of the portal vein is usually present in the rabbit near the point of entry into the liver, which it is advisable to tie.) The inferior vena cava was then tied just below the entry of the hepatic veins. The inferior vena cava was cut above the diaphragm, the stomach and intestines cut away, and the liver excised along with the diaphragm. The vessels were then washed out with 100 to 150 c.c. of 0·9 % saline solution. A cannula was next inserted into the superior vena cava. The liver was transferred in an evaporating basin to the perfusion apparatus, the connections made, and perfusion commenced. The perfusion fluid used was oxygenated Ringer solution (Locke formula). It was found that a perfusion pressure of 9—11 cms. of this solution gave a steady stream from the inferior vena cava. The outflow measured from 90—100 c.c.'s. per minute. If there were no venous obstruction there was practically no leak from the liver vessels: if a temporary obstruction occurred the liver swelled up and a leak commenced. Any slight leak of this nature was soon rectified and the transuded fluid added to the venous outflow. Some trouble was experienced with frothing and it was found advisable to use as small an amount of saline to wash out the vessels as was consistent with the removal of the contained blood. Prolonged washing out of the liver vessels increases the frothing.

200 mgrs. of parahydroxyphenylethylamine hydrochloride were first circulated through and through the liver in this way. At the end of one hour and two hours samples were taken and tested physiologically. Whereas the original fluid circulated produced a considerable rise of blood-pressure, the sample taken at the end of the first hour's perfusion was almost inactive and that taken at the end of the second hour was quite inactive. The fluid throughout the perfusion gave a good Millon

reaction and towards the end of the perfusion became faintly acid. From the perfusion fluid we were able to isolate parahydroxyphenyl-acetic acid.

With the aim of determining the quantitative relationship of the change, 500 mgrs. of the hydrochloride of the amine were perfused for three hours, and small quantities of alkali added from time to time as the alkalinity of the perfusion fluid fell off. At the end of this time the original perfusion fluid was withdrawn and fresh Ringer substituted. This was allowed to circulate for some time. The two lots of perfusion fluid were worked up together.

No parahydroxyphenylethylamine could be detected in the fluid but 310 mgrs. of crystalline parahydroxyphenylacetic acid were isolated. In other words 70 % of the maximum possible yield of the acid was obtained from the perfusion fluid alone.

From a number of other experiments it seems that a fair sized rabbit's liver can transform about 120 mgrs. of parahydroxyphenylethyl-amine into the acid every hour, yielding about 100 mgrs. of oxyphenyl-acetic acid. Thus 750 mgrs. of the hydrochloride of the amine perfused for one hour yielded 90 mgrs. of acid, while the same amount perfused under similar conditions for two hours yielded 190 mgrs.

The isolation and identification of the parahydroxyphenylacetic acid were carried out along the same lines as those adopted for urine. The process is, however, much easier with the comparatively simple Ringer solution as compared with the highly complex and pigmented urine. The combined perfusion fluids were rendered faintly acid with dilute acetic acid and boiled. The coagulated protein was removed by filtration and the filtrate concentrated by evaporation in vacuo on a water bath to about 40 c.c. The acid solution was then extracted with ether until the ethereal extract gave only a feeble reaction with Millon's reagent. The ethereal extracts were combined, washed with a very small quantity of water and the solvent evaporated off, when the acid was obtained slightly pigmented, but well crystalline and melting at 143—5°. When it was mixed with synthetic parahydroxyphenylacetic acid, m.p. 148°, the mixture melted at 146—7°.

It was thought possible that this change might be brought about by liver extracts but experiments in this direction all gave negative results. 200 mgrs. of parahydroxyphenylethylamine were incubated with finely divided cats' liver for six hours without antiseptics. No parahydroxy-phenylacetic acid could be detected and 80 % of the amine was recovered from the emulsion unchanged.

200 mgrs. of parahydroxyphenylethylamine were incubated with pounded rabbits' liver in the presence of chloroform for six days and no oxyphenylacetic acid could be detected. Another experiment was performed in a similar manner but the incubation period was prolonged to three weeks and the same result was obtained.

The perfusion experiments show that parahydroxyphenylethylamine is readily transformed by the liver into parahydroxyphenylacetic acid. This change may be quantitative if the perfusion be continued for a sufficient length of time. It is thus clear that the portion of the absorbed amine which is dealt with by the liver is transformed into the corresponding acid. Since any formation of parahydroxyphenylethylamine in the alimentary canal must necessarily be slow it appears improbable that any considerable quantity can pass into the general circulation unchanged. The change in all probability involves two steps

(1) Replacement of the NH_2 group by OH.
(2) The oxidation of the resultant alcohol to the acid.
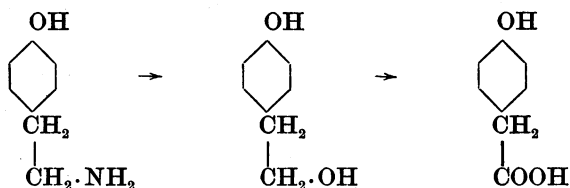
We have, however, no experimental evidence in support of this intermediate step.

In our feeding experiments the symptoms of sympathetic stimulation proved that when a fairly large dose is given by mouth a portion of the amine does reach the general circulation. We have therefore endeavoured to determine the fate of this portion. For this purpose we have performed further perfusion experiments on organs innervated by the sympathetic system since the physiological action is a clear indication that some reaction occurs at these points.

B. *Perfusion of isolated uterus.* A rabbit's uterus was perfused after Kurdinowsky's method [14]. In such a small organ the rate of circulation of the perfusion fluid is necessarily slow and as parahydroxyphenylethylamine is a potent vaso-constrictor, considerable difficulty was experienced in conducting a successful experiment. The perfusion fluid (400 c.c. Ringer solution) which contained 150 mgrs. of the amine circulated very slowly through the viscus for $2\frac{3}{4}$ hours. At the end of this

time oedema appeared and the perfusion was discontinued. We were able to isolate 8 mgrs. of crystalline oxyphenylacetic acid from the perfusion fluid. The small yield is in all probability largely due to the slow rate of perfusion. It seems clear, however, that plain muscle either of the uterus or the blood vessels supplying that organ can transform parahydroxyphenylethylamine into parahydroxyphenylacetic acid.

An interesting contrast is met with in the case of perfusion of the lung. 300 mgrs. of parahydroxyphenylethylamine were perfused through a rabbit's lungs for 1¾ hours. In this experiment there was a free flow through the organ, which increases, up to a certain point, the probability of a good yield of metabolic products; and yet no trace of oxyphenylacetic acid could be detected in the perfusion fluid.

It appears to us to be probable that the mucous membrane of the uterus on the one hand, and the cells of the lung alveoli on the other, can have but little influence on the result of an experiment of this nature, and that the results are the expression of the activity of plain muscle; in the one case that of the uterus itself or the blood vessels supplying it, and in the other that of the blood vessels of the lung. The results suggest that plain muscle having a rich motor sympathetic supply can effect the transformation of amine to acid; while plain muscle having a poor or no sympathetic supply (lung vessels see Brodie and Dixon[15]) is unable to do so.

C. *Perfusion of the isolated heart.* Our third series of perfusion experiments was conducted upon isolated hearts of rabbits, cats, and one dog. The results were uniformly in the one direction, whether a large dose of parahydroxyphenylethylamine was given at the commencement of the perfusion and allowed to circulate for one or two hours, or whether a series of small doses was given at ten minute intervals. In the former case the large dose at the beginning of the experiment throws a great strain upon the heart, and the beat, after a period of greatly increased frequency and force, becomes irregular. In the latter the heart continues regular for a much greater length of time and is nearer the normal. The result is, however, the same. We found that the greater part of the added base was destroyed but that there was no corresponding amount of oxyphenylacetic acid formed. In fact, ether extraction of the acid perfusion fluids gave practically no residue and no Millon reacting substance. The hearts were also examined for amine and acid but the results were also negative. Further we were unable to detect the presence of any substance which could be considered to be a product of the katabolism of the amine, and we believe that the base is

completely destroyed or broken down into quite simple compounds. This necessitates the break up of the benzene ring and the method by which this is effected in the body is unknown.

Cardiac muscle, therefore, although capable of dealing with para-hydroxyphenylethylamine does not appear to metabolise it along the same path as the liver or plain muscle. It was possible to show that parahydroxyphenylacetic acid was not an intermediate product in the degradation of the parahydroxyphenylethylamine molecule. A number of experiments were performed in which *p*-hydroxyphenylacetic acid (as Na salt) was perfused for varying lengths of time through the heart. It was always possible to recover the greater part of the acid unchanged. Some irregularity of heart beat was always noticeable in these experiments and the sodium salt of the acid appeared to exert a toxic influence. In one experiment 150 mgrs. of parahydroxyphenylacetic acid were administered in a series of small doses during two hours' perfusion of a dog's heart. Twenty-five minutes after the last dose had been given the perfusion fluid was examined for the acid and 100 mgrs. of pure *p*-oxyphenylacetic acid were recovered unchanged. In another experiment 70 mgrs. of acid were recovered from the perfusion fluid, to which 100 mgrs. had been added, and the whole perfused through and through a rabbit's heart for one and a half hours.

This distinction in the metabolic processes of plain and cardiac muscle is by itself of considerable interest, and we are of the opinion that this different method of metabolism will help to account for the comparatively small amount of oxyphenylacetic acid excreted in the urine in our feeding experiments with parahydroxyphenylethylamine. It is doubtful, however, if it will account for the whole of the difference between the amount of amine given by the mouth and the amount of acid excreted in the urine.

Having shown that parahydroxyphenylethylamine was readily transformed into oxyphenylacetic acid in the body it became of interest to determine whether methylation of the amino group would influence the direction of the metabolic process involved or the ease with which it is brought about.

We have tested this point with two different substances:

      (1)   The mono-methyl derivative.

      (2)   The di-methyl derivative.

      (1)   Parahydroxyphenylethylmethylamine.

For this base we are indebted to Mr G. S. Walpole[16]. As we had only a small amount at our disposal our experiments were limited

to liver perfusions. We found that the base was transformed into parahydroxyphenylacetic acid by the rabbit's liver but that the rate of destruction of the base was considerably slower than that of the primary amine. Thus 200 mgrs. perfused for two hours yielded only 65 mgrs. of acid. We have already shown that the yield of acid from parahydroxyphenylethylamine is about 100 mgrs. per hour.

(2)   Parahydroxyphenylethyldimethylamine or hordenine.

With this substance we have performed two sets of experiments

(*a*)   Feeding experiments.
(*b*)   Perfusion experiments on rabbits' livers.

In both sets of experiments the results are very similar. 1·0 gm. hordenine sulphate was given by mouth in a gelatine capsule to a dog. No symptoms were noticed beyond nausea and one slight attack of vomiting half an hour after the administration. The urine of the next 36 hours gave a vivid Millon reaction. This was found to be due to (1) a small amount of ether soluble acid, which was too small to isolate and identify but which was in all probability oxyphenylacetic acid; and (2) unchanged base. We were able to isolate with some difficulty 50 mgrs. of base in crystalline form. This merely accounts for about 7 % of the hordenine administered; the remainder apparently disappeared.

(*b*)   Perfusion of the liver gives very similar results. A trace of ether soluble acid, probably oxyphenylacetic acid, a somewhat small proportion of unchanged base, and considerable destruction.

Exp.   500 mgrs. of hordenine sulphate were perfused through a cat's liver for 2½ hours. The perfusion fluid was acidulated, boiled, filtered, and evaporated to dryness. The residue was extracted with water until the extracts no longer gave a Millon reaction, the aqueous solution acidified with HCl, and completely extracted with ether. The ethereal solution on evaporation yielded a very small quantity of residue from which was isolated a milligram or so of a substance giving an intense Millon reaction. This substance was most probably oxyphenylacetic acid.

The aqueous solution after extraction with ether was made alkaline with sodium carbonate and thoroughly extracted with ether. The ethereal solution was taken to dryness and extracted with benzene and this on evaporation yielded 35 mgrs. of crystalline hordenine.

From the foregoing experiments it seems clear that whereas the amino base *p*-hydroxyphenylethylamine is readily converted by the organism and more especially by the liver, into oxyphenylacetic acid, the corresponding methylamino base is much less readily metabolised in this direction, and with the dimethylamino base the difficulty is still further increased and little or no change takes place along this path.

On the other hand the introduction of methyl groups in this manner appears to render the base less resistant to complete destruction since in both feeding and perfusion experiments the amount of base which cannot be accounted for is progressively greater as we pass from the primary to the tertiary base.

Summary.

(1)   Parahydroxyphenylethylamine is converted in part in the body into parahydroxyphenylacetic acid.

(2)   The surviving liver can effect this change.

(3)   Plain muscle of the uterus can effect this transformation. Plain muscle of the lung vessels cannot effect this transformation.

(4)   The isolated heart causes complete destruction of the amine.

(5)   Parahydroxyphenylethylmethylamine is less readily converted into oxyphenylacetic acid than the primary amine; and hordenine, the tertiary base, still less readily than the secondary base. The proportion of base unaccounted for is on the other hand greatest with the tertiary and least with the primary base.

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