135. THE RATE OF REMOVAL OF AMINES FROM THE BLOOD

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(*Received 1 October 1941*)

PREVIOUS work on the amine oxidase has suggested that this enzyme is concerned primarily in the detoxication of amines produced by bacterial action in the gut [Richter, 1938; Richter & Tingey, 1939]. Amines are readily destroyed by preparations of the enzyme *in vitro*; but no information has hitherto been available as to the rate at which amines are detoxicated by this enzyme and removed from the blood *in vivo*.

The rate of detoxication of amines in the body is of particular significance in pharmacological work, in which it is desirable to know for how long after administration amines are still active in the blood. Further information as to the rate of inactivation of amines *in vivo* also appeared desirable in view of the suggestion that certain pathological conditions may be due to autointoxication by amines [Alvarez, 1924; Looney, 1924; Stewart, 1929; Quastel, 1937]. Amines are known to be produced not only by the putrefactive bacteria occurring normally in the intestine, but also by pathogenic strains, such as those of *Bact. typhosum* and *C. welchii*, which may invade the tissues, and amines have been isolated from pus [Kaunitz & Wacek, 1935; Lachampt, 1938; Gale, 1940]. Several of the amines produced by bacteria, such as putrescine, cadaverine and the lower aliphatic amines, are relatively non-toxic; but β-phenylethylamine, tyramine and the higher aliphatic amines show marked sympathomimetic activity and are toxic even in very small quantities. In the normal healthy individual these amines are readily destroyed by the amine oxidase in the liver, intestine and other tissues [Bhagvat *et al.* 1939]; but it has been suggested that in pathological conditions the detoxication may be deficient, giving rise to an abnormally high level of amines in the blood [Quastel, 1937].

Quastel & Wheatley [1933] have obtained evidence of the way in which amines might act in exerting their toxic action; but there is no evidence at present available as to the actual concentration of these amines in the blood in normal or pathological conditions. It appeared that for the further investigation of this problem there are required (*a*) a highly sensitive method for estimating traces of amines in the blood, and (*b*) a method of testing the efficiency with which amines are detoxicated *in vivo*. An attempt has therefore been made to devise such methods and to use them for obtaining further data on normal subjects.

The estimation of amines in blood

The usual methods for estimating 'amino-nitrogen', such as those of Folin or of Van Slyke, are not sufficiently sensitive for estimating the traces of amines that might be present in blood, and as they apply to amino-acids and amino-compounds in general a preliminary separation of the amines from the other amino-derivatives would be necessary. The colorimetric method using the amine

picrate [Richter, 1938] is sufficiently sensitive and is specific for amines or amino-compounds that are extracted from aqueous solution by organic solvents. Preliminary experiments showed that specimens of normal blood frequently contain a substance that gave a strong amine reaction when tested by this method. The substance was non-volatile and was not decomposed by nitrous acid; it was identified as a phospholipin, probably lecithin, which reacts by virtue of the quaternary amino-group in the choline residue. Added lecithin behaved in a similar manner. The method was therefore modified in order to avoid interference from lecithin.

Principle of method. The amines are extracted from whole blood by shaking with light petroleum and an excess of K_2CO_3, which liberates the free amines and at the same time exerts a salting out action. The loss of amine through combination with traces of aldehydes in the blood to form Schiff's bases, which may become significant at the lower amine concentrations, is reduced by adding a small amount of tyramine, which is not extracted. The amines are separated from phospholipins by taking them into a small volume of *N* H_2SO_4 saturated with NaBr; the phospholipins remain in the light petroleum solution. The amines are then taken back into light petroleum and picric acid is added to form the picrates, which give a strong yellow colour in chloroform solution.

Procedure. The blood was collected in a tube containing potassium oxalate as anticoagulant and 10 ml. blood were transferred to a 30 ml. centrifuge tube and stirred with about 5 mg. solid tyramine hydrochloride. Light petroleum (10 ml. of B.P. 80–100°) and saturated K_2CO_3 solution (5 ml.) were added and the tube was shaken vigorously for 3 min. The emulsion was broken down by alternately centrifuging and stirring with a thick glass rod: 7 ml. of the light petroleum layer were then pipetted into a 10 ml. centrifuge tube containing 0·5 ml. of a solution of *N* H_2SO_4 which had been saturated with NaBr. The mixture was shaken for 4 min., centrifuged and the light petroleum layer discarded. The acid was washed by adding 3 ml. fresh light petroleum, which, after shaking for 3 min. and centrifuging, was again discarded. The amines were now taken back into organic solvent by adding 2·4 ml. light petroleum and 0·2 ml. 40 % KOH and shaking for 6 min. After centrifuging, the light petroleum layer was transferred to a clean, dry test tube; it was mixed with 2·4 ml. chloroform and the colour was developed by adding 0·1 ml. 2 % picric acid in chloroform. The colour was then compared with a series of standard solutions prepared by adding known amounts of β-phenylethylamine or *iso*amylamine to samples of blood (up to 50 μg./ml.) and treating them in the same manner. For some purposes the direct comparison by eye in test-tubes of uniform bore was sufficiently accurate, but greater accuracy was obtained by using a photoelectric absorptiometer with Ilford spectrum violet filters. A calibration curve giving amine concentration × absorptiometer reading was first prepared with the standard solutions; the amine concentration in solutions of unknown strength could then be obtained from this curve. A blank using 10 ml. distilled water was repeated with each set of estimations.

Discussion of method. It is essential that the light petroleum and chloroform used should be free from amines or other compounds giving a colour with picric acid, so that a good blank is obtained. Different samples of light petroleum and chloroform were found to vary greatly in this respect: one specimen of technical light petroleum gave a better blank than the 'A.R.' The solvents must therefore be tested and purified if necessary by extracting with acid before use. In view of the sensitivity of the method to traces of acids or alkalis it was found necessary to clean all the apparatus used with cleaning mixture and to pay special attention

to the thorough rinsing and drying of the test tubes and pipettes. The tubes were kept stoppered as much as possible to prevent evaporation of the petroleum. Clean corks covered with cellophane were generally used for shaking. The partition of the amines between the aqueous and organic phases is affected considerably by the proteins present in blood; amine solutions in blood and not aqueous solutions were therefore used in preparing the standards for colorimetric comparison. Five specimens of normal blood which had been taken for transfusion and which were used in the preliminary experiments showed no trace of amine when tested by this method: one specimen of ox blood and one of guinea-pig blood were also found to be free from amines.

The method is specially adapted to the estimation of amines such as β-phenylethylamine or the higher aliphatic amines which are produced by the putrefactive bacteria and which are readily soluble in organic solvents. The sensitivity differs for different amines according to their solubilities and molecular weights; the following figures give an approximate estimate of the colour intensities obtained with aqueous solutions containing 20 μg./ml. of a number of different amines, expressed in terms of the concentration of β-phenylethylamine (μg./ml.) which gave the same intensity of colour:

Trimethylamine	16	Tryptamine	3
*iso*Amylamine	20	Tyramine	0
Putrescine	0	*sec*Butylamine	20
Mescaline	4	Ephedrine	8
Methylamine	0	Creatinine	0

The method of taking the amine into a small volume of acid enabled a concentration of the amine to be effected. The sensitivity of the method for a given amine depended on the volumes taken for the different extractions. The volumes given were chosen arbitrarily as being convenient, but the sensitivity was increased by using 1 ml. of light petroleum instead of 2·4 ml. for the final extraction and adding 1 ml. of chloroform. Under these conditions an addition of 1 μg./ml. of β-phenylethylamine to normal blood could be readily detected.

The rate of detoxication of amines in vivo

Some indication of the rate of detoxication of β-phenylethylamine was obtained in experiments reported previously [Richter, 1938] in which the amine was taken by mouth and the rate of excretion of phenylacetic acid in the urine was measured. The amount of acid excreted in 2¼ hr. corresponded to 40 % and in 4½ hr. 62 % of the theoretical amount expected from the 300 mg. amine which were taken. This showed that the rate of detoxication is rapid; but the rate of absorption of the amine in the gut and the rate of coupling of the phenylacetic acid with glutamic acid (it is excreted as phenylacetylglutamine) come in as unknown factors which would have to be determined before the true rate of detoxication could be deduced from experiments of this kind.

Blood-pressure test

A further indication of the rate of detoxication was obtained by measuring the duration of the blood-pressure rise after β-phenylethylamine had been administered intravenously. The systolic and diastolic blood pressures were measured by the auscultatory method with a sphygmomanometer applied to the brachial artery. The subject for the test was a patient (62 kg.) with hysterical symptoms, but in whom a careful examination had failed to show any physical

abnormalities. The solution for injection contained β-phenylethylamine hydrochloride (5 % base) in distilled water and it was sterilized by keeping at 100° for 1 hr. Blood pressures taken before and after the intravenous injection of 1·5 ml. (75 mg. base) are recorded in Fig. 1.

The systolic blood pressure rose sharply to 220 mm. Hg after the injection, but was back to normal again within $2\frac{3}{4}$ min. This suggested that the rate of detoxication of β-phenylethylamine might be very rapid; it would be of the order of 26 mg./kg. body weight/hr., if the amine were all destroyed when the blood pressure returned to normal.

The blood pressure test is very easy to carry out and might perhaps be used clinically as an indication of amine oxidase function; but it is doubtful whether one is justified in assuming that the return of the blood pressure to normal coincides with the complete detoxication of the amine. It appeared that a more

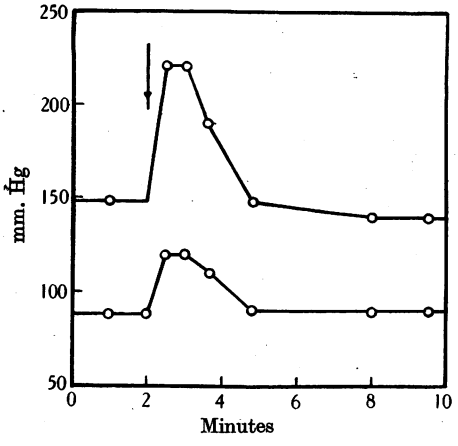

Fig. 1

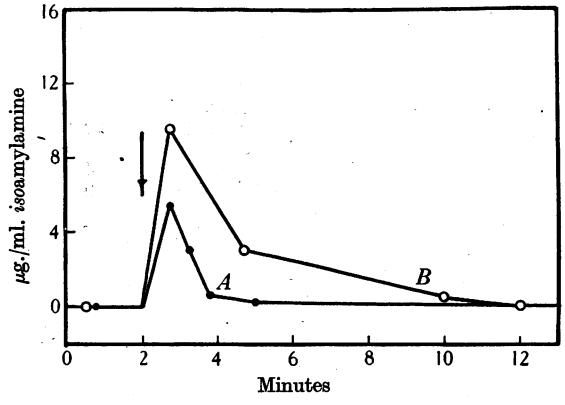

Fig. 2

Fig. 1. Blood pressure, systolic above and diastolic below, after the intravenous injection of 75 mg. β-phenylethylamine at the time marked by the arrow. Subject (62 kg.).

Fig. 2. Concentration of *iso*amylamine in the blood after the intravenous injection of 500 mg. *iso*amylamine at the time marked by the arrow in subjects *A* (60 kg.) and *B* (66 kg.).

reliable indication of the rate of detoxication would be obtained by estimating directly the amount of amine remaining in the blood at different times after an intravenous injection of an amine.

Amine removal test. β-Phenylethylamine is too toxic for it to be administered in sufficiently large doses to be estimated conveniently in the blood. *iso*Amylamine, which is relatively non-toxic, was found to be more suitable for this purpose. A solution of *iso*amylamine hydrochloride containing 12·5 % of the base was sterilized for intravenous injection by heating at 100° for 1 hr. In preliminary trials with gradually increasing doses the dose was raised to 4 ml. of the 12·5 % solution (500 mg. base) for subjects of about 60 kg. and with this dose the reaction was never unpleasantly severe.

Procedure. In carrying out this test 4 ml. 12·5 % *iso*amylamine were introduced into a 5 ml. syringe and several clean, dry 10 ml. syringes were kept ready for withdrawing samples of blood. With the subject in the reclining position 10 ml. of blood were taken from the left arm. The amine solution was injected into the median cubital vein of the right arm and at intervals of 40 sec., 100 sec.,

3 min. and 10 min.; after the injection, further 10 ml. samples of blood were withdrawn from the left arm. The blood was collected in tubes containing potassium oxalate and the amount of *iso*amylamine remaining in the blood was estimated as above.

Five tests were carried out as described on subjects who had been treated for mild nervous disorders but who were physically normal so far as could be ascertained at the time of the tests: the results of two such tests are shown in Fig. 2. The time required for the amine to be removed from the blood ranged from about 2 to 8 min.; this would indicate a rate of removal of the order of 60–250 mg. *iso*amylamine/kg. body weight/hr.

An indication of the fate of the amine was obtained with one subject in whom it was observed that the blood smelt strongly of *iso*amyl alcohol in the samples taken 100 sec. or more after the injection. The removal of amine from the blood in the first few seconds immediately following the injection must be due in part to the diffusion of the amine out of the blood and into the tissues, but the final removal of all the amine from the blood must be ascribed to an irreversible detoxication. The amine is first oxidized and deaminated by the amine oxidase and the resulting aldehyde is then oxidized further to the acid or converted into a mixture of the corresponding alcohol and acid by the aldehyde oxidase and aldehyde mutase.

The efficiency of the amine-detoxicating mechanism is inversely proportional to the area under the amine removal curve (Fig. 2). This gives a method of measuring the amine oxidase function, which might be expressed as

$$\frac{\text{mg. amine administered}}{\text{area} \times \text{kg. body weight}}.$$

The amine oxidase function was of the order of 1 to 2 in the normal subjects who have been examined. In view of the sensitivity of the method of estimating blood amines, extreme accuracy cannot be claimed for this method of measuring the amine oxidase function; but the method would appear to be adequate for demonstrating any gross defect in the amine-detoxicating mechanism.

Discussion

The idea that toxic amines might play a part in disease derives mainly from the work of Barger and Dale, who first examined the physiological properties of a large series of amines and demonstrated that they are produced in putrefactive processes. Autointoxication by amines has been suggested to explain the hyperglycaemia and other symptoms observed in intestinal obstruction. It has also long been known that the symptoms observed clinically in certain types of nervous disease can be reproduced artificially by administering amines such as mescaline or other β-phenylethylamine derivatives, and this has led to the view that toxic amines might play a special part in these disorders [Richter & Lee, 1941].

Apart from a few attempts to estimate blood histamine and adrenaline, no figures have hitherto been available for the normal level of amines in the blood. There has also been no information as to the efficiency of the normal mechanism for detoxicating amines *in vivo*. The results obtained by the methods now described indicate that human blood normally contains less than 1 part per million of amines of the β-phenylethylamine or *iso*amylamine types, and that the body is normally capable of detoxicating about 4–15 g. *iso*amylamine per hour. This is much greater than the rate at which amines are likely to be formed

under normal conditions by bacterial action in the gut and suggests that the body has a considerable capacity in reserve for detoxicating amines.

In a study of the reactions of 5 men who voluntarily induced constipation lasting for 90 hr., Donaldson [1922] observed the symptoms of coated tongue, foul breath, hyperglycaemia, impaired appetite, mental sluggishness, loss of attention, restlessness, irritability, depression, poor sleep, increased reaction time and headache. Donaldson concluded that these symptoms could not be attributed to toxic substances in the blood, since the symptoms were promptly removed by giving enemata, while he assumed that detoxication must be a relatively slow process. This assumption is now shown to be incorrect as far as amines are concerned, for injected amines are completely removed from the blood within a few minutes.

The method of estimating blood amines which has now been described applies to β-phenylethylamine and the higher aliphatic amines, but not to tyramine, histamine or amines insoluble in organic solvents. However, the conditions (acid medium) which favour the production of β-phenylethylamine and the aliphatic amines also favour the production of tyramine and histamine, which are likely to be present under conditions in which amines of the former type are found.

The amine oxidase function test indicates the efficiency of the detoxication of all the amines oxidized by this system; these include β-phenylethylamine, the aliphatic amines, tyramine and tryptamine, but not histamine. It is suggested that the amine oxidase function test and the other tests described should be used for the clinical investigation of conditions in which intoxication by amines is suspected.

Summary

1. A sensitive method is described for estimating amines in the blood. The method is capable of detecting 1 part per million of β-phenylethylamine or *iso*amylamine.

2. Specimens of normal ox blood, guinea-pig blood and human blood contained less than 1 part per million of amines when tested by this method.

3. The rate of detoxication of β-phenylethylamine administered intravenously in man was estimated from the time taken by the blood pressure to return to normal to be of the order of 26 mg./kg. body weight/hr.

4. The rate of detoxication of *iso*amylamine administered intravenously in man was estimated from the rate of removal from the blood to be of the order of 60–250 mg./kg. body weight/hr.

The authors wish to thank Prof. Nevin and Dr Maclay for their helpful interest, Dr MacIntyre for taking some of the blood specimens and the Rockefeller Foundation for supporting this investigation.

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