THE ISOLATION OF BASES FROM URINE CONCENTRATES

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In previous communications (Lockett[1,2]) the name base B was applied to a compound present in the concentrates of steam distillate from normal dog and human urine, which had the property of raising the blood pressure of chloralosed cats, when injected intravenously. Concentrates of base B gave a non-specific colour test (x) when picric acid in chloroform solution was added to the base dissolved in a mixture of toluene and chloroform. Attempts to separate a compound responsible for colour production from that having pressor effect, or to demonstrate more than one compound responsible for either property failed. The methods employed were numerous, and included a long series of adsorption experiments, the use of many different precipitants and solvents; some of these experiments have been summarised (Lockett[3]). The two tests, colour production and pressor effect, were therefore considered to indicate the presence of a single compound which was shown to be volatile in steam, soluble in water, alcohol, chloroform, benzene and toluene, but to be insoluble in ether and light petroleum. It was unstable to acid and was readily oxidised.

In the first part of the present communication an account is given of the separation of base B of dog urine as the reineckate. The second part deals with the isolation of degradation products which were obtained from base B concentrates of human urine, and not from the reineckate. Two basic degradation products, piperidine and pyrrolidine, were obtained in pharmacologically significant amount, and had pressor activity. It was therefore of interest to compare the pharmacological actions of base B concentrates with those of these two degradation products. The pharmacological evidence (Lockett [4]) showed that the pressor activity of base B concentrates could be distinguished, in a number of ways, from that of piperidine and pyrrolidine; it therefore supported the chemical evidence that these last two bases were only obtained from base B concentrates by degradation.

Methods

Preparation of base B concentrates. Two methods were used, and gave concentrates of approximately equal pressor activity and solid content per litre of original urine. The reineckate of base B separated more readily from concentrates prepared by method 2.

Method 1. This has been described under the heading of the preparation of concentrates of x-positive base (Lockett[3]). Nicotine was removed by adsorption on blood charcoal.

Method 2. The urine was brought to *p*H 11·6 (sodium hydroxide), and was submitted to continuous ether extraction for 24 to 30 hours. The extracted urine was distilled in steam, *p*H 12·0 (sodium hydroxide), and

the distillate was concentrated *in vacuo*, below 35°C., without the addition of acid. No blood charcoal was used.

Separation of the reineckate of base B from dog urine. Base B concentrates were prepared by method 2 from successive 3 l. fractions of dog urine. Each concentrate (vol. 3·0 to 4·0 ml.) was neutralised to litmus as external indicator with N/1 sulphuric acid. A 4 per cent. solution of ammonium reineckate was added until no further precipitation occurred, and the solution remained well coloured. After standing in the ice-chest for 48 hours, the crude reineckate was collected, washed by centrifugation, and dried over calcium chloride; the collected reineckate was recrystallised from methyl alcohol.

The degradation of compounds present in base B concentrates from normal human urine. Incomplete oxidation with potassium permanganate in acid solution. Successive fractions of base B concentrates from 413 l. of normal human urine were treated with N/10 potassium permanganate in 1 per cent. sulphuric acid at room temperature, the oxidation being carried to a point just beyond the end of the rapid phase (2 to 3½ hours). The manganese dioxide was filtered off, and the filtrates were combined and distilled in steam from acid solution; the distillate gave a precipitate with silver nitrate, in the presence of nitric acid. After cooling the flask contents were extracted with ether; the ether extracts were dried over anhydrous sodium sulphate, the ether was removed by distillation, and there remained a small quantity of ether-soluble oil. The ether-extracted acid aqueous solution was freed of ether, cooled in ice, made alkaline with sodium hydroxide, and distilled in steam, dilute hydrochloric acid traps being used to prevent the escape of highly volatile bases. The distillate was acidified with hydrochloric acid, the trap contents were added, and the whole was evaporated to dryness under reduced pressure. The residue of crude hydrochloride was several times treated with alcohol and again evaporated to dryness. A search was made, without success, for non-volatile bases remaining in the flask contents after distillation from alkaline solution.

Complete oxidation with potassium permanganate in alkaline solution. The procedure differed little from that described for oxidation in acid solution. The concentrates were derived from 306 l. of urine. Oxidation with N/10 potassium permanganate in 2 per cent. sodium carbonate solution was carried to completion. The combined filtrates were acidified with sulphuric acid, and were worked up as described above under oxidation in acid solution.

RESULTS

Precipitation of base B as the reineckate. Neutral one-thousandfold concentrates of base B, prepared by method 2 from dog urine, yielded a crystalline powder when treated with ammonium reineckate. The crude reineckate had pressor activity, and its separation was attended by complete loss of pressor activity from the supernatant fluid (Fig. 1). Approximately 4 mg. of crude reineckate (chromium 14·1 per cent.) was obtained per l. of original urine. One-fifth of this weight represented

base. Since base B concentrates contained approximately 1 mg. of solid per l. of urine, there were other pharmacologically inert compounds present in base B concentrates which were not precipitated by ammonium reineckate.

Eight recrystallisations of the reineckate from 24 l. of dog urine yielded a compound which composed 90 per cent. by weight of the crude solid.

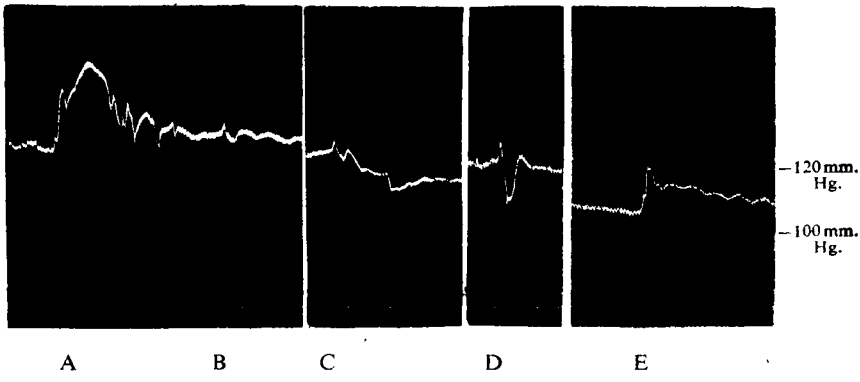

FIG. 1. Responses of the arterial blood pressure of a cat under chloralose anæsthesia, wt. 2·8 kg., to intravenous injection:—(A) 4·5 ml. of base B concentrate, equivalent to 0·7 l. of urine; (B) 5 ml. of the filtrate after the addition of 0·5 ml. of 4 per cent. ammonium reineckate solution to 4·5 ml. of base B concentrate; (C) 0·5 ml. of 4 per cent. ammonium reineckate solution diluted to 5 ml.; (D) 10 ml. of 10 per cent. ethyl alcohol; (E) 5 mg. of crude reineckate dissolved in 1 ml. of ethyl alcohol and diluted rapidly to 10 ml. with 0·9 per cent. sodium chloride solution just before injection. Each solution contained 0·9 per cent. of sodium chloride. Time marker, 30 sec.

The pressor activity of this material was less than that of the crude reineckate. Although five further recrystallisations did not significantly alter the analyses, the purified reineckate obtained at the end had only a depressant action on the blood pressure (Fig. 2).

Found: Crude reineckate Cr, 14·1 per cent.; 5th recrystallisation Cr, 13·8 per cent. 8th recrystallisation Cr, 13·9 per cent. 13th recrystallisation C, 22·31; H, 4·05; N, 26·8; Cr, 13·9 per cent. C_3H_9N requires C, 22·28; H, 4·14; N, 25·92; Cr. 13·76 per cent. $C_6H_{16}N_2$, dibasic, requires C, 22·28; H, 3·98; N, 25·99; Cr, 13·79 per cent.

The crystals from methyl alcohol were purple-red and grew in clusters. They were almost totally insoluble in water.

The isolation of degradation products from base B concentrates. The study, by degradation, of compounds present in base B concentrates, prepared either by the first or second method, from human urine, has consistently yielded simple volatile bases which can readily be separated in crystalline form. Before degradation these bases could not be crystallised from base B concentrates. The bases isolated after degradation were ammonia, dimethylamine, piperidine, pyrrolidine, and two unidentified compounds.

Several volatile pressor bases have already been isolated from normal urine:—nicotine (Dingemanse and Freud[5]), *iso*amylamine (Bain[6]), a nicotine compound (Lockett[1]), piperidine (Euler[7]). These compounds

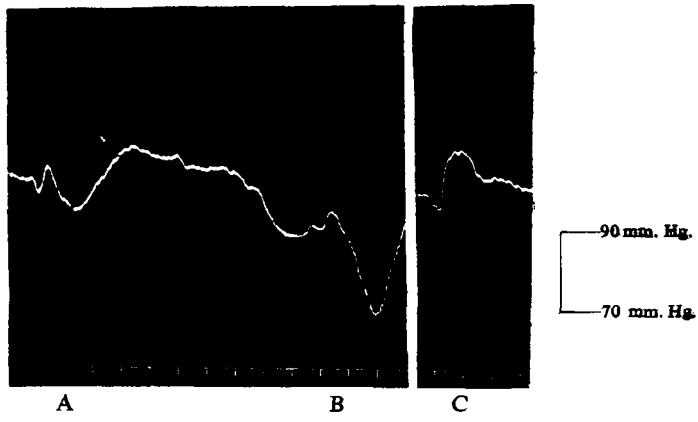

FIG. 2. Responses of the arterial blood pressure of a cat under chloralose anæsthesia, wt. 2·1 kg., to 4 mg. of crystals dissolved in 1 ml. of ethyl alcohol, and diluted to 10 ml. with 0·9 per cent. sodium chloride solution immediately before injection:—(A) 8th recrystallisation of the crude reineckate; (B) 13th recrystallisation of the crude reineckate; (C) crude reineckate. Time marker, 30 sec.

are soluble in ether and would therefore have been extracted from concentrates prepared by method 2. Using concentrates prepared by each method, in six experiments, the addition of piperidine, dimethylamine, and ammonia in concentrations varying from 1 in 1000 to 1 in 10,000, and of nicotine in a concentration of 1 in 20,000 to measured aliquots of the original urine, yielded concentrates which did not differ significantly in pressor activity from concentrates obtained from untreated aliquots of the same urine. In no case could the compound be recovered from the treated concentrates. The added bases had therefore been removed by the processes involved in the preparation of base B concentrates by either method.

Incomplete oxidation of base B concentrates in chlorine-free sulphuric acid solution yielded a mixture of bases, an ether-soluble oil, and hydrochloric acid. The ether-soluble oil had a pungent smell, and some volatility in ether vapour; it soon deposited floccules on standing; the addition of alcohol to its ether solution resulted in a white floccular precipitate, insoluble in water, acids, alkalis, chloroform, but with a very low solubility in ether. After drying in a high vacuum at room temperature the compound was white and waxy; sodium fusion showed the presence of C and H, and absence of N, halogen, P and S. Found C, 84·16; H, 13·19 per cent.; mol. wt. by depression of the freezing-point of camphor, 940.

The crude hydrochloride, which contained all the bases, was separated into alcohol-soluble and alcohol-insoluble fractions. The alcohol-insoluble hydrochloride, after recrystallisation, was white, mildy deliquescent, and decomposed slowly with the appearance of sublimation from about 280°C. The whole was converted to picrate, and was purified by recrystallisation. The base was liberated from the pure picrate and a picrolonate, nitrate, phosphomolybdate, and platinichloride were prepared. The analyses and melting-points corresponded closely with those of ammonia derivatives, and the mixed melting-points were satisfactory. This base formed nearly three quarters of the total weight of crude hydrochloride obtained.

1. *Identification of ammonia.*—Picrate from alcohol-insoluble hydrochloride, yellow needles, m.pt. 291°C. Ammonium picrate similarly prepared, m.pt. 291°C. with decomposition in each case. Found: C, 29·77; H, 2·39; N, 22·6 per cent. Calculated: C, 29·27; H, 2·44; N, 22·77 per cent.

Picrolonate. Purple sheen. Decomposed 280°C. Ammonium picrolonate had the same appearance, and decomposed at 280°C. Found: C, 43·38; H, 4·1; N, 25·3 per cent. Calculated: C, 42·71; H, 3·9; N, 24·9 per cent.

Nitrate. Transparent needles, m.pt. 168°C. Ammonium nitrate m.pt. 169°C., mixed m.pt. 168°C.

The crude alcohol-soluble hydrochloride was strongly deliquescent; the whole was converted to picrate, and fractional crystallisation was carried out. Great difficulty was encountered from oil formation, and the separation was not quantitative; five well defined fractions were obtained and recrystallised to constant melting-points. From three of these picrates other derivatives were prepared which led to their identification as piperidine, pyrrolidine, and dimethylamine. The fourth and fifth picrates were obtained in rather small amount, 25 and 16 mg.; they have not yet been identified.

2. *Identification of piperidine.* Picrate m.pt. 145°C. Piperidine picrate m.pt. 147°C. Mixed m.pt. 145°C. Found: C, 41·55; H, 4·1; N, 17·9 per cent. C, 41·73; H, 4·57; N, 18·1 per cent. previous experiment. C, 41·96; H, 4·52; N, 18·3 per cent. previous experiment. Calculated: C, 42·04; H, 4·46; N, 18·1 per cent.

Picrolonate. Brownish yellow, m.pt. 248°C. with decomposition. Piperidine picrolonate decomposed 248° to 250°C. Found: C, 51·47; H, 5·3; N, 21·6; N-Me. 0 per cent. C, 51·2; H, 5·16; N, 21·0; N-Me. 0 per cent. previous experiment. C, 50·05; H, 5·48; N, 19·6 per cent. previous experiment. Calculated: C, 51·58; H, 5·45; N, 20·01 per cent.

Platinichloride, from aqueous solution, m.pt. 201°C. Piperidine platinichloride m.pt. 201°C. Mixed m.pt. 201°C.d.

3. *Identification of dimethylamine* Picrate m.pt. 155°C., yellow flattened plates. Dimethylamine picrate m.pt. 156°C. Mixed m.pt. 156°C. Found: C, 35·69; H, 3·12; N, 21·15 per cent. C, 35·2; H, 3·4; N, 20·8 per

cent. previous experiment. Calculated: C, 35·3; H, 3·6; N, 20·5 per cent.

Platinichloride. Found: C, 9·32; H, 2·28; N, 6·22 per cent. Calculated: C, 9·6; H, 3·2; N, 5·8 per cent.

Picrolonate, yellow, m.pt. 252°C. Dimethylamine picrolonate m.pt. 252°C. Mixed m.pt. 252°C., each with decomposition.

4. *Identification of Pyrrolidine.* Picrate m.pt. 112°C. Pyrrolidine picrate m.pt. 112°C. Mixed m.pt. 112°C. Found: C. 40·39; H, 5·04; N, 18·0 per cent. Calculated: C, 40·0; H, 4·0; N, 18·67 per cent.

Picrolonate, brick red, decomposed from 260°C. to 264°C. Found: C, 50·62; H, 5·25; N, 21·0; N-Me, 0 per cent. Calculated: C, 50·16; H, 5·08; N, 20·9 per cent.

Gold chloride, m.pt. 206°C.d. Pyrrolidine 206°C.d.

5. *Data for unidentified base.* Picrate, pale yellow needles, m.pt. 136°C. Found: C, 41·2; H, 4·26; N, 18·5; N-Me, 8·8 per cent. Calculated for $C_9H_{20}N_2$, and 2N-Me, dibasic, C. 41·04; H, 4·24; N, 18·24; N-Me, 9·4 per cent.

Picrolonate, yellow orange, decomposing 258°C. to 263°C. Found: C, 50·67; H, 5·6; N, 21·4 per cent. Calculated: C, 50·88; H, 5·26; N, 20·46 per cent.

6. *Data for unidentified base.* Picrate, golden needles, decomposing at 281°C. Found: C, 42·47; H, 4·33; N, 18·1, 18·9; N-Me, 6·8 per cent.

Oxidation of base B concentrate by N/10 potassium permanganate in 2 per cent. sodium carbonate solution, when carried to completion, yielded hydrochloric acid, and two volatile bases. The first base was present in large amount, and was identified as ammonia; the second, isolated as a picrate which was eventually crystallised, was present in small amount, and was identified as dimethylamine.

7. *Identification of Ammonia.* Picrate, long yellow needles, m.pt. 291°C. Mixed with ammonium picrate, m.pt. 291°C. Found: C, 29·55; H, 2·48; N, 23·0 per cent. Calculated: C, 29·27; H, 2·44; N, 22·77 per cent.

Picrolonate, purple sheen, decomposing at 280°C. Resembled ammonium picrolonate both in appearance and in decomposition point.

Acetate, m.pt. 87°C. Mixed m.pt. 87°C.

Phosphomolybdate. Greenish yellow crystalline powder.

8. *Identification of dimethylamine.* Picrate, m.pt. 155°C. Mixed with dimethylamine picrate m.pt. 155°C

9. *Identification of chlorine.* The precipitate with silver nitrate solution in the presence of nitric acid, was washed with water and alcohol. and was dried *in vacuo,* and was analysed. Found: Cl., 25·2 per cent. AgCl requires Cl, 24·9 per cent.

Pressor activity of pyrrolidine and piperidine. Since piperidine and pyrrolidine both have pressor activity, the identities of picrates 2 and 4 were further established by comparison with those of piperidine and pyrrolidine in respect of their effect on the blood pressure of a cat under

chloralose anæsthesia. In equating these picrates it was found necessary to wait 15 minutes between each injection, since repeated injections of pyrrolidine, in the same dose, at short intervals, sometimes produced decreasing responses.

7·2 mg. of picrate 2 equated very nearly with 7·0 mg. of piperidine picrate, both given in a volume of 3·0 ml. when similarly administered to a cat, wt. 2·4 kg. (Fig. 3A and B), 4·6 mg. of pyrrolidine picrate gave

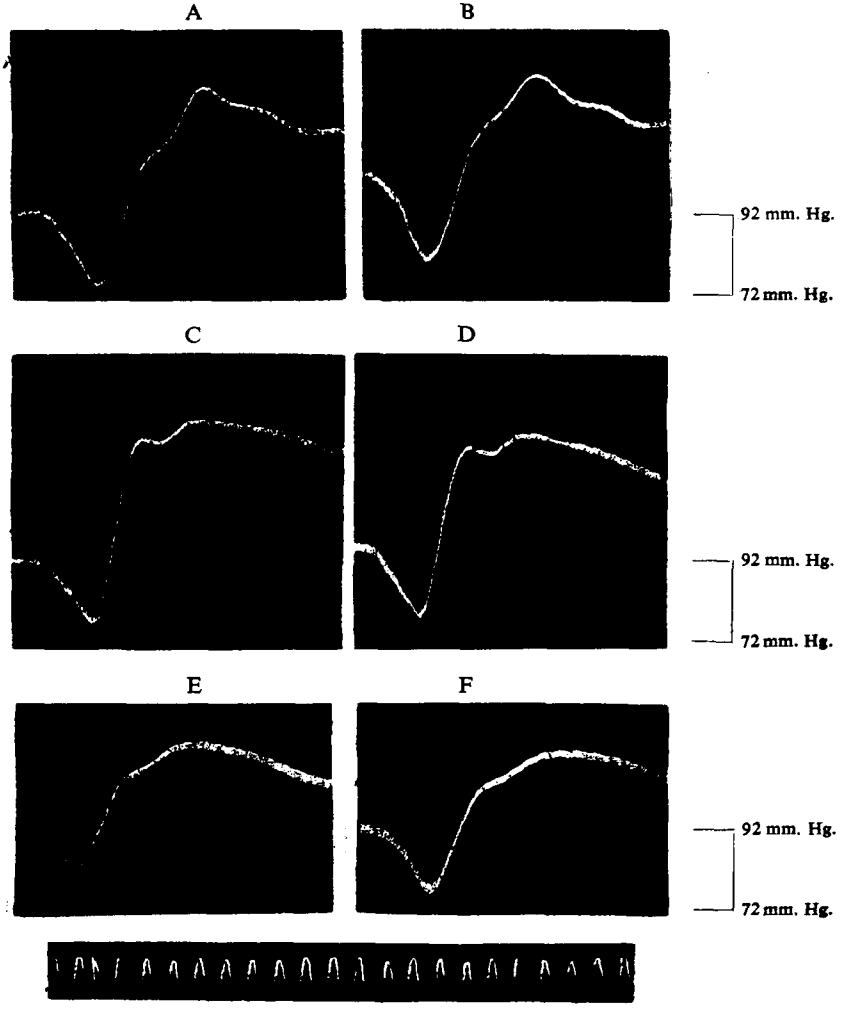

FIG. 3. Responses of the arterial blood pressure of a cat under chloralose anæsthesia, wt. 3·6 kg., to intravenous injection:—(A) 7·2 mg. of picrate 2; (B) 7·0 mg. of piperidine picrate; (C) 4·6 mg. and (D) 4·2 mg. of pyrrolidine picrate; (E) 4·5 mg. and (F) 4·1 mg. of picrate 4. Time marker, 30 secs.

a response comparable with that which followed 4·5 mg. of picrate 4, each in a volume of 4·5 ml. (Fig. 3C and D) and 4·2 mg. of pyrrolidine

picrate equated with 4·1 mg. of picrate 4, each in a volume of 4·0 ml. (Fig. 3E and F).

Discussion

The interest of these results is centred on the high degree of pressor activity which must be attributed to base B. Since the purified reineckate accounted for at least 90 per cent. of the crude solid, and 5 mg. of crude material were obtained per l. of original urine, 0·5 mg./l. of urine is the maximum weight that could be ascribed to base B. Of this 0·5 mg. it is unlikely that more than 20 per cent. by weight could have represented free base. The weight of base B required to produce a small or moderate rise of arterial blood pressure when injected intravenously into a cat, cannot be greater than 100 μg. That the estimated dose may prove to be considerably greater than the real is suggested by the results recorded in Figure 2, and the analyses listed; in the absence of any change in the chromium content, pressor activity was succeeded by depressor action as the result of repeated recrystallisation. The depressor compound isolated has been found to give the colour test *x* which previously appeared to be inseparable from the pressor action of base B concentrates, and was therefore used as an aid to the separation of base B. This depressor compound may prove to be a simple diamine; its pharmacological action is compatible with this view.

The occurrence of piperidine and pyrrolidine as degradation products of compounds present in base B concentrates is interesting, and indicates that further evidence relating to the intermediary metabolism of these heterocyclic compounds may be obtained from a study of urinary trace bases.

Summary

1. Base B has shown to be a compound of high pressure activity, and a depressor compound, empirical formula $C_6H_{16}N_2$ has been isolated as the reineckate from base B concentrates.

2. Piperidine, pyrrolidine, dimethylamine and ammonia have been obtained as degradation products from compounds present in base B concentrates, prepared from human urine.

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