EXCRETION OF PIPERAZINE SALTS IN URINE

BY

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Urinary excretion of piperazine has not been widely studied owing to the lack of a quantitative method of analysis in biological fluids. A solution of potassium bismuth iodide gives a red precipitate with piperazine, and was used by Zimmermann (1901) for the qualitative demonstration of the drug. Nessler's reagent gives a heat-soluble precipitate; the picrate, chloroaurate, and platinate are also characteristic. Piperazine is precipitated by tannic acid, and can also be identified in urine by shaking a strongly alkaline sample with benzoyl chloride and determining the melting-point of the final product after crystallization in alcohol. Valseth and Wickström (1954) employed the titration of the mono-periodate; this is not sensitive enough for biological determinations. A potentiometric method is described by Davies *et al.* (1954), but this has not yet been applied to urine. All of these methods require preliminary treatment of the urine or specialized apparatus, and are not suitable for routine clinical analyses.

The present paper describes a colorimetric method, using 1:2-naphthoquinone-4-sulphonic acid (Folin's amino-acid reagent) for the quantitative determination of piperazine in urine. It is a modification of a procedure originally devised by Dr. Eileen Short (private communication), and, although it is not specific for piperazine, the method gives accurate results, and is suitable as a practical laboratory procedure involving the minimum number of manipulations. Amino-acids and other substances present in the urine also give coloured derivatives with the reagent, but the effect of interfering substances can be eliminated by using internal standards in a manner similar to that of Harris and Raymond (1939) for the determination of nicotinic acid. The method has been employed to study the excretion rates in human subjects after oral doses of piperazine. The results include a small number of excretion values from Africans infected with ascarides.

Determination of Piperazine in Urine

Reagents.—2% sodium tetraborate (borax), $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, in distilled water. 96% ethyl alcohol. 0.5% 1:2-naphthoquinone-4-sulphonic acid (specially purified sodium salt, Folin's amino-acid reagent, B.D.H.); the solution must be used within two hours of preparation. Standard piperazine: a solution of piperazine citrate containing 20 $\mu\text{g.}/\text{ml.}$ (calculated as hexahydrate) in distilled water.

Method.—Label three test-tubes R.B. (reagent blank), U. (unknown), and U.S. (unknown+standard). Dilute the urine under examination 1:25 with distilled water. Pipette 1 ml. of the diluted urine into tubes U. and U.S. Pipette 2 ml. of distilled water into tube R.B. and 1 ml. of distilled water into tube U. Pipette 1 ml. of standard piperazine

FIG. 1.—Method of calculation of piperazine concentration. Readings of U. (35.5 $\mu\text{g.}$) and U.S. (55.0 $\mu\text{g.}$) are joined and produced backwards to cut the axis and give the amount of piperazine present (36.5 $\mu\text{g.}$) in the original 1 ml. of diluted urine.

(20 $\mu\text{g.}$) into tube U.S. Add to all three tubes 2 ml. of 2% borax and mix. Add 5 ml. of 96% alcohol, mix, add 1 ml. of 0.5% 1:2-naphthoquinone-4-sulphonic acid. Mix by inversion of the tube. Allow to stand 10 minutes and read U. and U.S. in a photoelectric colorimeter at 480 $\text{m}\mu$, using R.B. as the blank for each determination.

Calculation.—From the two readings obtained (U. and U.S.) calculate the piperazine present by reference to a standard curve prepared by using pure solutions of piperazine. Take these values as ordinates and the amount of piperazine added (0 and 20 $\mu\text{g.}$) as abscissae. The

FIG. 2.—Cumulative excretion rates of piperazine citrate, phosphate, and adipate in human volunteers after oral dosage. Broken lines are rates in Africans infected with ascarides.

straight line obtained by joining the two points is produced backwards and cuts the axis at a distance from the origin which represents the amount of piperazine present in the original 1 ml. of the diluted urine (Fig. 1). With the apparatus used in this laboratory all known solutions were found to give results which were 5 μ g. too small. A constant correction of 5 μ g. was therefore made for each determination.

Dilution of the Urine and Blank Values.—Dilution of the urine 1:25 brings the blank value due to naturally occurring substances within readable limits, and these values are eliminated by the procedure described above. Piperazine standard curves follow Beer's law for samples containing amounts up to 100 μ g., and then the curve flattens slightly for amounts up to 200 μ g. The amount of piperazine present in the diluted sample must be between 0 and 200 μ g. for the actual determination. Higher concentrations of piperazine give rise to rapid precipitation, and, if necessary, the urine must be further diluted.

Colour Reaction.—Upon the addition of the final reagent a reddish-brown colour develops rapidly during the first 20 seconds and reaches its optimum in 10 minutes. After this period the colour increases slowly until precipitation takes place; hence it is essential to take the readings at exactly 10 minutes after the addition of the colour reagent. Reproducible results are given at room temperature (20° C.); higher temperatures increase the colour value in all tubes, and it is therefore necessary to prepare a standard curve appropriate to the temperature of the analytical conditions. The 1:2-naphthoquinone-4-sulphonic acid reagent gives consistent readings with a given quantity of piperazine for a period of two hours after preparation. After this time it slowly darkens and eventually precipitates, giving rise to erroneous results. The recovery of piperazine added to normal urine was found to be satisfactory.

Excretion of Piperazine after Oral Administration in Man

A brief report of the urinary excretion of salts of piperazine by human volunteers was given by Standen *et al.* (1955).

Materials and Methods.—The preparations used were as follows: Piperazine phosphate and piperazine citrate (B.W. & Co.), compressed tablets each containing 500 mg. of piperazine calculated as the hexahydrate. Piperazine adipate ("entacyl"), tablets each containing 251 mg. of piperazine calculated as the hexahydrate. For each excretion study the subject was given tablets equivalent to 3 g. of piperazine hexahydrate together with 300 ml. of water. The dose was given three hours after breakfast. Ten healthy adult volunteers were used for each piperazine salt. In addition excretion rates were determined in four Africans infected with ascarides. Three of these received phosphate and one adipate. Urine was collected at each emptying of the bladder, and the volume and the time were noted. No preservatives were used. Determinations of piperazine were made by the method described, in batches of six samples at a single run. This was found to be the maximum number that could be handled at any one time to keep within the time limit of 10 minutes for development of colour imposed by the method.

Results.—Fig. 2 shows the cumulative excretion of piperazine phosphate, citrate, and adipate. It is clear from these graphs that: (1) there is a wide variation in the rate at which piperazine is excreted by different individuals; (2) the rate of excretion by Africans infected with ascarides was of the same order as the rates in uninfected Europeans; and (3) there was no significant difference between the rates of excretion of piperazine citrate, adipate, and phosphate.

Summary

A method is given for the quantitative analysis of piperazine in urine using 1:2-naphthoquinone-4-

sulphonic acid. The method has been used to study the urinary excretion rates of human subjects after oral administration of piperazine phosphate, citrate, and adipate. No significant difference between the rates of excretion of the three salts was observed.

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ACTION OF NICOTINE ON THE HEART

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The usual view of the action of nicotine is that it is a ganglionic stimulant. Almost all students are taught that Langley painted a solution of nicotine on sympathetic ganglia to discover the anatomical distribution of the cells giving rise to post-ganglionic fibres. When a certain effect, such as pilo-erection, followed the application of nicotine to one ganglion he knew that cells giving rise to fibres which innervated the pilomotor muscles were present in that ganglion.

The increase in heart rate which occurs on smoking a cigarette has usually been explained as a consequence of nicotine stimulating (1) the ganglia of the sympathetic chain from which the nervi accelerantes arise, and (2) perhaps also the cells of the adrenal medulla, liberating adrenaline. If this explanation were correct nicotine would not be expected to have any stimulant action on the isolated heart. The only ganglia believed to be present in the heart itself are the parasympathetic ganglia where the fibres of the vagus make synaptic connexion with the short post-ganglionic fibres. The effect of nicotine on these ganglia would be to slow the heart.

A few years ago, however, our colleague Kottogoda (1953) excised the rabbit heart and separated the atria from the rest of the cardiac tissue, suspending them in a bath so that their contractions could be recorded on a drum. When nicotine was added to the bath he did not observe inhibition, but he observed a mixed effect which was at first inhibitory and then became excitatory. By adding to the bath atropine to exclude the inhibition, he was able to study the quite powerful excitatory action which nicotine then produced. Nicotine caused an increase in rate and amplitude such as is illustrated in Fig. 1 a. The concentrations of nicotine used to produce the effect were, expressed in terms of nicotine base, from 3×10^{-6} g./ml. upwards.

The question then arose to what this stimulant action of nicotine was due. There seemed to be two possi-