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Quantitative method for determining aminofurantoin†

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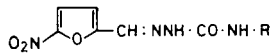
A quantitative colorimetric procedure for the estimation of aminofurantoin is described. The red colour of the Schiff base produced by the reaction of dimethylaminobenzaldehyde with aminofurantoin, is stable after a period of 40 min for a further 20 min and can be measured at 582 mμ. Maximal colour is developed at room temperature (20°) under acid conditions (pH 2·0). This technique was found suitable for the determination of aminofurantoin in tissue homogenates. The formation of aminofurantoin as a probable metabolite of nitrofurantoin was investigated but its production was not confirmed.

IN view of the extensive use of nitrofurantoin (5-nitro-2-furfurylidene-amino hydantoin) as a urinary antiseptic, it is surprising that the metabolic fate within mammalian tissue is still uncertain. Paul, Ellis & others (1960) discussed the probable metabolites produced by the physiological degradation of substituted semicarbazone derivatives of 5-nitro-2-furfurylidene (I).



I

The compounds appear to be biochemically degraded by hydrolysis of the side chain to give nitrofuraldehyde followed by oxidation to nitrofuroic acid (Paul, Austin & others, 1949; Paul & others, 1960) and by the reduction of the nitro-group followed by ring cleavage. Evidence suggests that the intermediate hydroxylamine is formed but the aminofurans could not be isolated.

Studies on the degradation of nitrofurantoin by bacterial systems have shown that a single stage breakdown occurs involving the simultaneous reduction of the nitro-group and the cleavage of the furan ring (Beckett & Robinson, 1956). However, Ebetino, Caroll & Geur (1962) found that the catalytic reduction of nitrofurantoin produced 1-(5-aminofurfurylidene-amino)hydantoin, which was quite stable and showed *no ring cleavage*. Although aminofurantoin is a postulated intermediate in the metabolic degradation of nitrofurantoin, there is still no direct evidence for its formation in mammalian tissues.

The difficulties inherent in isolating unchanged nitrofurantoin and its metabolic products from urine or tissue homogenate have made it tedious to separate and characterize the metabolites. Therefore a simple colorimetric procedure for the quantitative estimation of aminofurantoin in tissue homogenates has been developed in an attempt to facilitate the characterization of this compound as a possible metabolite; the need for preliminary tedious chromatographic separation is thereby avoided.

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† Aminofurantoin is the accepted trivial name for 1-(5-amino-2-furfurylidine-amino)hydantoin.

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The method developed by Bratton & Marshall (1939) for the quantitative estimation of aromatic amines using naphthylethylenediamine was found to be unsuitable for the determination of aminofurantoin. Therefore, the method of Venkataraman, Venkataraman & Lewis, (1948) has been investigated as a possible alternative.

The red colour produced by the interaction of Ehrlich's reagent (*p*-dimethylaminobenzaldehyde 1% in ethanol) with aminofurantoin is sensitive, unaffected by the presence of nitrofurantoin and is sufficiently stable for accurate measurements to be made.

METHODS

Ehrlich's reagent (1% *p*-dimethylaminobenzaldehyde (BDH) in ethanol) (100 ml) containing concentrated hydrochloric acid (1 ml) was used to develop the colour which was measured on a Unicam SP800 at 582 mμ. Aminofurantoin (50 mg) was dissolved in *NN*-dimethylformamide (1 ml) and this was diluted with water to give a stock solution containing 100 μg/ml.

Dilutions of the stock solution of aminofurantoin were prepared to give concentrations between 2 and 15 μg/ml. The aminofurantoin solution was adjusted to pH 1·8 by the addition of Clark & Lubbs buffer (3·5 ml to 1 ml of the aminofurantoin solution); Ehrlich's reagent (0·5 ml) was added to the acidified aminofurantoin (4·5 ml) and the mixture was allowed to stand at room temperature (20°) before reading the absorption at 582 mμ.

The procedure was modified to study the effect of time (colour determined after 10 min intervals), temperature (20–48°) and pH 1·0–2·0. In each case a solution containing 20 μg/ml of aminofurantoin was used. The results are presented in Figs 1 and 2.

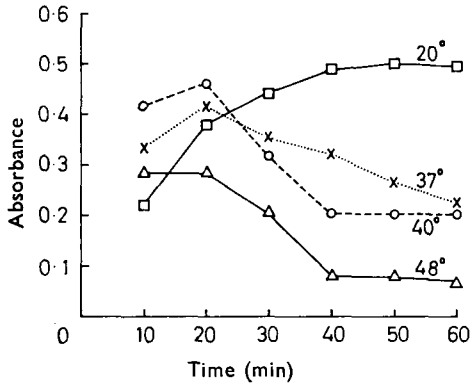

FIG. 1. Effect of temperature and time on the colour development (582 mμ) resulting from the interaction of aminofurantoin with Ehrlich's reagent.

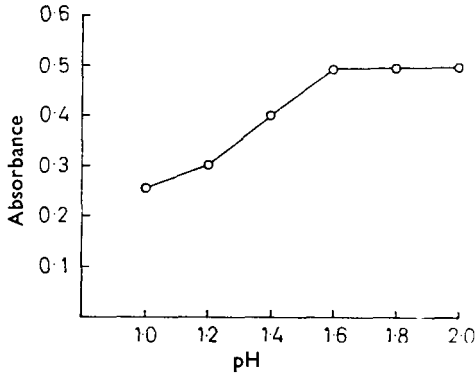

FIG. 2. Effect of pH on the colour development resulting from the interaction of aminofurantoin with Ehrlich's reagent.

Recovery of aminofurantoin from tissue homogenates. Aminofurantoin solutions (1·5 ml) were added to freshly homogenized rat liver (5 g in 10 ml of phosphate buffer, pH 7·4) (2 ml) to give final concentrations of 4–15 μg/ml. Trichloroacetic acid 10% in water (10 ml) was added to the homogenate preparation and the precipitated protein removed by centrifugation. Dilute ammonium hydroxide solution was added to correct the pH of the supernatant to pH 1·8. The above process was repeated replacing the homogenate with water. The volumes of the final solutions were adjusted to 15 ml, Ehrlich's reagent (0·5 ml) was added to 4·5 ml aliquots and the colour determined at 582 mμ. The percentage recovery of aminofurantoin at different concentrations is presented in Table 1.

TABLE 1. RECOVERY OF AMINOFURANTOIN FROM BIOLOGICAL FLUIDS

Concentration of added aminofurantoin		Concentration of "recovered" aminofurantoin µmole/ml			% Recovery		
µg/ml	µmole/ml	Water	Homo-genate	Urine	Water	Homo-genate	Urine
4	0·019	0·019	0·019	0·019	100%	100%	100%
7·5	0·036	0·036	0·034	0·034	100%	97%	97%
10	0·048	0·048	0·047	0·046	100%	97%	95%
15	0·072	0·072	0·068	0·064	100%	98%	95%

Incubation of nitrofurantoin with tissue homogenates. Nitrofurantoin and *p*-nitrobenzoic acid solutions (1 ml) were added to separate samples of a guinea-pig liver homogenate as described for aminofurantoin above, to give concentrations of 1 μmole/ml. The homogenates were fortified by the addition of a co-factor solution (2 ml) containing NADPH (1·1 μmole), glucose-6-phosphate (19 μmole), riboflavine (2·5 μmole) and nicotinamide (1·5 μmole) in phosphate buffer (pH 7·4).

The preparations were incubated at 37° for 1 hr under nitrogen, the reactions stopped with trichloroacetic acid and the colour developed as described above. *p*-Aminobenzoic acid (1·25 μmole) was produced in

the reaction mixture containing nitrobenzoic acid as substrate whereas no aminofurantoin could be detected in that preparation containing nitrofurantoin.

Incubation of aminofurantoin with tissue homogenates. Aminofurantoin (1 μmole) was added to freshly homogenized rat liver as described above. The homogenate was incubated at 37°, samples were removed at 10 min intervals, and the aminofurantoin content determined (see Fig. 3).

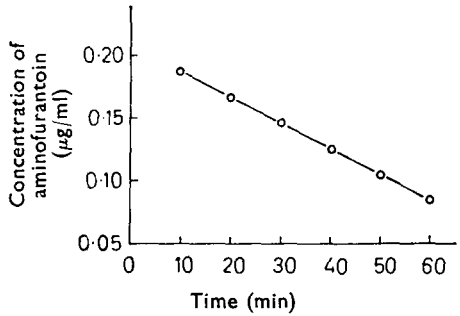

FIG. 3. Decrease in aminofurantoin concentration on incubation with rat liver homogenate.

Attempted recovery of aminofurantoin from urine. Furadantin (200 mg) was given orally to two men whose urinary pH was maintained at 5·0 by ammonium chloride (1 g orally every 3 hr). A 4 hr urine sample was collected and analysed for aminofurantoin content as described. No aminofurantoin was detected although nitrofurantoin was shown to be present by a spectroscopic examination of a urine extract.

Results

The colour development was found to be: (i) time dependent, reaching a maximum at 20° after a period of 40–60 min (see Fig. 1), (ii) temperature dependent, the optimal temperature being 20° (see Fig. 1), (iii) pH dependent, requiring an acidic pH optimal at 1·8 (see Fig. 2).

The absorption by the Schiff base formed during the reaction was shown to obey the Beer Lambert law below concentrations of 15 μg/ml, and was sensitive enough to detect concentrations as low as 2 μg/ml.

A mixture of Ehrlich reagent and rat liver homogenate under the conditions described produced no measurable absorption at 582 mμ, and excellent recoveries were obtained of aminofurantoin added to an homogenate (see Table 1).

However, upon incubating aminofurantoin with the homogenate a significant loss was observed as shown in Fig. 3.

Discussion

Ehrlich's reagent forms a Schiff base with aminofurantoin having an absorption maximum at 582 mμ which is stable under defined conditions of temperature and pH, and whose intensity is quantitatively related to

low concentrations of the aminofurantoin. The colour development with aromatic amines reported by Vankataraman & others (1948) was maximal at a pH of 2·0–2·2. However, using aminofurantoin a pH of 1·8 was required for maximum colour development.

Good recoveries were obtained from rat liver homogenate; quantities of 2 μg/ml being detected by the method. The technique described appears to offer a reasonably sensitive and convenient method for the detection of aminofurantoin in the presence of biological material.

Although the system used was shown to reduce *p*-nitrobenzoic acid to *p*-aminobenzoic acid it was not possible to demonstrate the formation of aminofurantoin as a reduction product from nitrofurantoin. However, it was shown that aminofurantoin is broken down at an appreciable rate under the conditions used, and therefore if it is formed by the action of nitroreductase on nitrofurantoin it is probably immediately converted by some mechanism to another reaction product. Therefore it was not suprising that aminofuranotin was not detected in the urine of persons receiving nitrofurantoin, even though the urinary pH favoured maximum excretion of any basic material.

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