PLASMA-SALICYLATE CONCENTRATIONS AFTER SMALL DOSES OF ACETYLSALICYLIC ACID

By M. J. H. SMITH

From the Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, S.E.5

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THE measurement of plasma concentrations of both free and acetylated salicylate after the oral administration of 5 to 15 grains of aspirin is of interest in studies of the effects of such doses on pain thresholds[1] and also in the investigation of the absorption of acetylsalicylic acid. A number of methods are available for the determination of plasma-salicylate concentrations in the range 10 to 40 mg./100 ml. but these procedures are not easily applicable to lower concentrations. The development of a method[2] for measuring plasma concentrations from 0 to 10 mg./100 ml. of the closely related substance, gentisic acid, suggested the possibility of modifying this procedure to the determination of similar salicylate and acetylsalicylate concentrations.

The present method is based on the observation that both salicylic acid and acetylsalicylic acid are quantitatively extracted from acidified plasma by ethyl acetate and completely removed from the organic solvent by 1 per cent w/v sodium bicarbonate solution. Two samples of plasma are extracted in this manner and an aliquot of each bicarbonate extract is taken, in one of these aliquots the acetylsalicylic acid is converted to salicylic acid by hydrolysis with sodium hydroxide and the salicylic acid content of both solutions is estimated by means of the reaction with an aqueous solution of the Folin-Ciocalteu phenol reagent[3]. A blue colour having an absorption maximum at 670 mμ develops on the addition of sodium

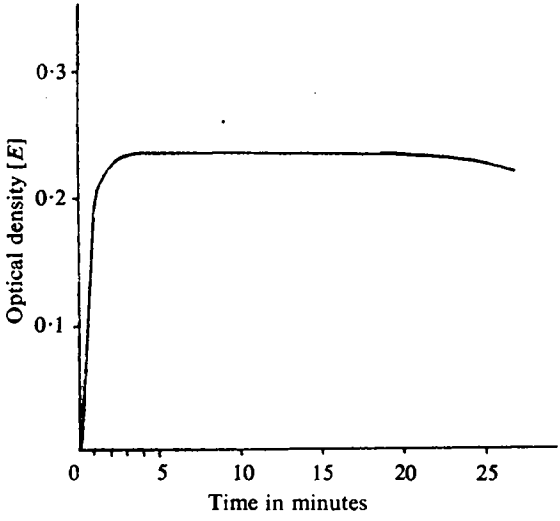

FIG. 1. Rate of development and fading of Folin-Ciocalteu blue colour at room temperature.

hydroxide; the colour intensity reaches a maximum after 5 minutes then begins to fade after 20 minutes and is reduced to about 90 per cent. of the maximum intensity after 2 hours (Fig. 1).

The absorption density of the coloured solution was found to be proportional to the concentration of salicylic acid up to 10 mg./100 ml. and the method gave recoveries of 95 per cent. of both salicylic acid and acetylsalicylic acid from plasma. A calibration curve (Fig. 2) may be constructed from a series of solutions of salicylic acid ranging in concentration from 0 to 10 mg./100 ml.

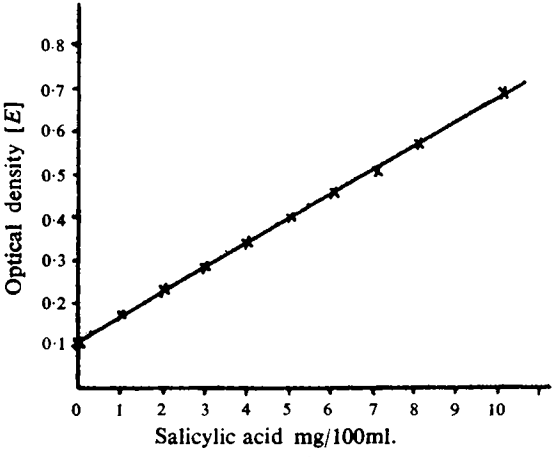

FIG. 2. Calibration curve of salicylates in distilled water, using the blue colour given by the Folin-Ciocalteu reagent in alkaline solution. The colour intensities were measured in a Hilger Spekker absorptiometer using an Ilford spectrum red filter, No. 608.

The colour reaction with the Folin-Ciocalteu reagent depends on the reduction by phenols of hexavalent molybdenum and tungsten to coloured products of lower valence. The method therefore estimates not only salicylic acid but also metabolic products of salicylic acid such as gentisic and salicylic acids, which also contain free phenolic groups. Gentisic acid forms only 4 to 8 per cent. of salicylate metabolites[4] and may be neglected when small plasma-salicylate concentrations are being measured. About 50 to 60 per cent. of ingested salicylic acid is excreted in the urine as the glycine conjugate, salicyluric acid[4], but Brodie, Udenfriend and Coburn[5] reported that only negligible amounts of salicyluric acid were present in plasma, and Lester, Lolli and Greenberg[6] reported that protein-free filtrates of blood of subjects receiving salicylates did not show an increase in amino-acid content after hydrolysis.

Two types of salicylic acid glucuronides occur in urine[7], an alkali-labile form, in which a molecule of glucuronic acid is conjugated in an ester linkage with the carboxyl group of salicylic acid, and an alkali-stable form, in which there is an ether linkage between the glucuronic acid and the hydroxyl group of salicylic acid. Kapp and Coburn[4] estimated that about 25 per cent. of salicylate was excreted in combination with glucuronic acid, and most of this fraction was readily hydrolysed by alkalies suggesting that the ester-linked glucuronide

predominated. No information is available about the occurrence of these substances in blood. Free phenolic groups would not be liberated from ether-linked glucuronide occurring in plasma under the experimental conditions used in the present methods, but ester-linked glucuronide could liberate salicylic acid after alkaline hydrolysis.

Free plasma-salicylate concentrations obtained with the present method may therefore be taken to represent salicylic acid only and the differences between these concentrations and the values obtained after alkaline hydrolysis to represent acetylsalicylic acid plus any ester-linked glucuronide which may be present.

Samples of blood were collected from 8 healthy normal subjects at timed intervals after the administration of two 5 grain tablets of aspirin B.P. and plasma concentrations of free salicylic acid and acetylsalicylic acid measured in each sample. Opportunity was taken to compare the absorption of the official preparation with a proprietary preparation claimed to be more quickly absorbed. These tablets contain 5 grains of acetylsalicylic acid in an edible base and were chewed and not swallowed whole. Each subject received both preparations with at least two days interval and under similar conditions. The results which are given in Table I and Figure 3 demonstrate that this preparation was absorbed more rapidly than the official preparation.

TABLE I

Time (minutes)	Plasma-salicylate concentrations mg./100 ml. (Mean figures ; σ = standard deviation)			
	Free		Total	
	Tab. Aspirin B.P.	Proprietary	Tab. Aspirin B.P.	Proprietary
10	0·215	0·680	0·370	1·170
	σ = ·312	σ = ·474	σ = ·330	σ = ·658
20	0·400	1·640	1·180	2·361
	σ = ·408	σ = ·642	σ = ·730	σ = ·935
30	0·910	2·725	1·810	3·454
	σ = ·634	σ = ·941	σ = ·926	σ = 1·280
60	2·470	4·060	3·775	5·230
	σ = 1·180	σ = 1·210	σ = ·916	σ = 1·120

The individual results were analysed by means of Student's t-test and the figures for the free and total plasma-salicylate concentrations after the administration of the proprietary preparation differ significantly from the corresponding figures for Tab. Aspirin B.P., the values of P being less than 0·01 in each case.

These results demonstrate that after doses of 10 grains (0.65 g.) of acetylsalicylic acid, measurable plasma-concentrations of salicylic acid may be attained in 10 minutes and values up to 5 mg./100 ml. may be observed after 60 minutes. These results are in general agreement with other workers[6,8] who used a method of estimation involving extraction with ethylene dichloride and a final colour reaction with ferric nitrate[5].

Hanzlik and Presho[9] found unchanged acetylsalicylic acid in the urine of patients given the drug, but their analyses depended upon the difference in intensity of the colour reaction with iron before and after hydrolysis of the urine. Their conclusion that 25 per cent. of the salicylate was unchanged acetylsalicylate has been criticised[10] because the procedures employed could also cause hydrolysis of the salicyl glucuronides present. No acetylsalicylic acid could be detected in plasma by Smith, Gleason,

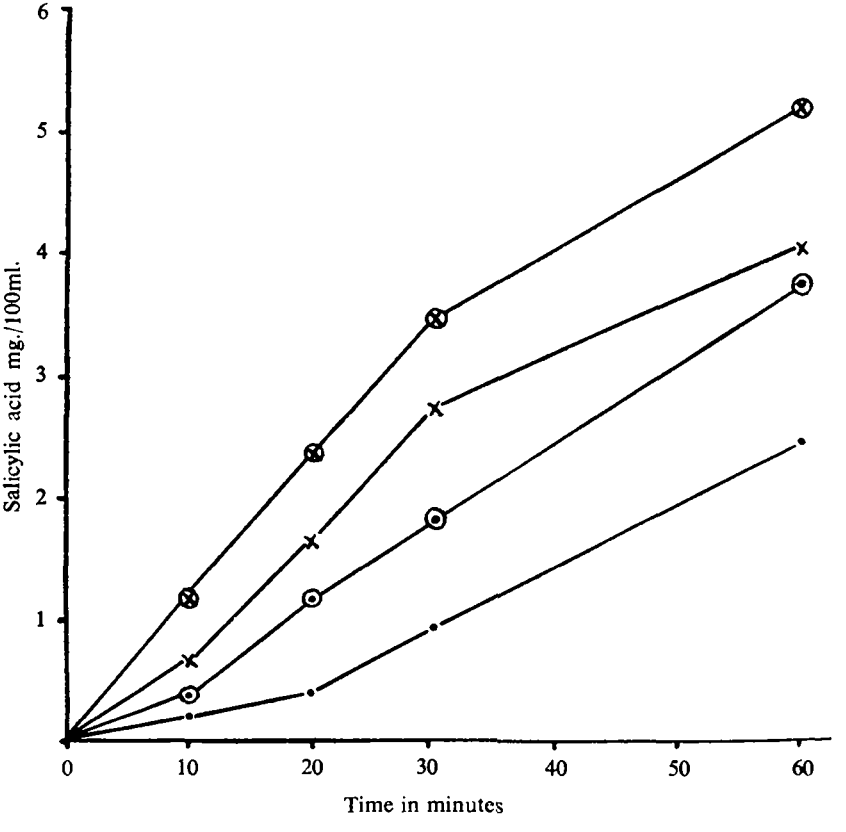

FIG. 3. Mean plasma concentrations of free and total salicylates after single doses of 10 grains of aspirin as Tab. Aspirin B.P. and the proprietary preparation.

● — Free plasma salicylates (Tab. Aspirin).
⊙ — Total plasma salicylates (Tab. Aspirin).
× — Free plasma salicylates (proprietary).
⊗ — Total plasma salicylates (proprietary).

Stoll and Orgorzalek[11] after a single dose of 2 g. of aspirin, but Lester, Lolli and Greenberg[6] reported that 25 per cent. of the salicylate in plasma after the administration of acetylsalicylic acid consisted of the unchanged ester. The present results indicate that the plasma-acetylsalicylate concentration parallels the plasma-salicylate concentration with time and

that one hour after the administration of 0·65 g. of acetylsalicylic acid approximately 30 per cent. of salicylates in the plasma are present as the unchanged acetylated form.

Experimental

Two tablets of aspirin B.P. were given to each subject and samples of blood were collected by venepuncture before, and at 10, 20, 30 and 60 minutes after the dose was administered. No dietary or fluid intake restrictions were imposed except that the drug was given at least 2 hours after a meal. The same procedure was followed with each subject with the second aspirin preparation at least 48 hours interval being allowed to elapse between the experiments in order to ensure that all the salicylate from the first experiment had been excreted.

Each sample of blood was analysed for free and total salicylates by the methods described below. Plasma from subjects who had not received salicylates gave a blue colour in excess of the reagent blank and the value obtained for the blood sample from each subject taken before the dose was given, was subtracted from the values obtained from the subsequent samples in order to correct for the individual variation.

Methods

(a) *Free plasma-salicylate concentrations.* 0·5 ml. of 6N hydrochloric acid is added to 2 ml. of plasma followed by 10 ml. of ethyl acetate. The mixture is shaken for 2 minutes and centrifuged for 5 minutes; 5 ml. of the ethyl acetate layer is removed and added to 5 ml. of 1 per cent. w/v sodium bicarbonate solution. The mixture is shaken and centrifuged; 4 ml. of the bicarbonate layer is removed and 0·5 ml. of distilled water followed by 1 ml. of Folin-Ciocalteu reagent (diluted 1 to 3 with distilled water), are added and mixed. After the addition of 1 ml. of 1·5N sodium hydroxide the solution is allowed to stand for 5 minutes and the absorption density measured against distilled water in a photoelectric absorptiometer using a 1 cm. cell and a filter transmitting maximally above 660 mμ. A Hilger Spekker photoelectric absorptiometer and an Ilford spectrum red filter No. 608 has been used in the present work.

(b) *Total plasma-salicylate concentrations.* The procedure is similar to that for free salicylate except that 0.5 ml. of 1.5N sodium hydroxide is added to 4 ml. of the bicarbonate extract instead of 0·5 ml. of distilled water. The mixture, which is contained in a ground glass-stoppered pyrex test tube, is placed in boiling water for 15 minutes and then removed and cooled. The Folin-Ciocalteu reagent and sodium hydroxide are added and the optical density measured as before.

Summary

1. Methods of estimation of plasma-salicylate and plasma-acetylsalicylate concentrations in the range 0 to 10 mg./100 ml. are described.

2. These methods have been applied to the estimation of plasma con-

centrations of these substances at timed intervals after the administration of a dose of 10 grains of aspirin to 8 healthy subjects.

3. The rates of absorption of equivalent doses of two preparations of aspirin have been compared.

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