Blood Levels of Aspirin Following the Ingestion of Commercial Aspirin-Containing Tablets by Humans

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Circulating whole blood levels of aspirin (acetylsalicylic acid) were determined by a method of analysis which instantly stopped enzymatic hydrolysis and yielded a negligible blank reading. It was shown that at 10 min. after ingestion of commercial tablets of buffered or unbuffered aspirin by humans, 50 per cent of the blood salicylic acid was unhydrolyzed aspirin; this decreased to 30 per cent at 20 min.

LEONARDS (1) has demonstrated that aspirin is absorbed unhydrolyzed from the intestinal lumen. Unhydrolyzed aspirin persists in blood for some time after its oral ingestion (2–4). The duration of analgesia in humans appears to parallel the plasma aspirin levels rather than the plasma total salicylic acid levels (5, 6). Analgesia corresponded with aspirin levels rather than salicylic acid (SA) in dogs injected with bradykinin (7, 8). Therefore, the probability exists that aspirin itself is a more protent analgesic agent than the product of its hydrolysis, SA (9).

Most measurements of salicylates have been carried out with plasma or serum rather than whole blood, utilizing the procedure of Brodie *et al.* (10) or a modification thereof. The value so obtained is not equivalent to the whole blood salicylate level but is greater because the amount of salicylate present in erythrocytes is quite low and "of the same order of magnitude as might be expected if the cell membrane were freely permeable to the salicylate contained in the plasma ultrafiltrate" (11). If such a condition does indeed exist, then the erythrocyte concentration of salicylate is inversely related to the degree of plasma binding. Davison and Smith (12) and Lester *et al.* (5) have shown that SA is bound extensively to bovine serum albumin, while acetyl salicylate shows "little or no interaction." Hence, it is possible that erythrocyte concentrations of acetylsalicylate could be much higher than those of salicylate. Therefore, plasma aspirin/salicylate ratios may differ from those of whole blood.

A second problem of analysis is due to the continuing enzymatic hydrolysis of aspirin during preparation of the plasma or serum when plasma or serum is used for analysis. Enzyme inhibitors proved unsatisfactory for this purpose.

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The purpose of this communication is to present a reproducible procedure for measuring total and hydrolyzed aspirin which eliminates the objections described above and to present results obtained with the method for actual commercial preparations which, although in essential agreement with other investigators (1–4), are quantitatively different.

EXPERIMENTAL

Determination of Free (Unacetylated) Salicyclic Acid (FSA) and Total Salicylic Acid (TSA).—The method described below is a modification of the method of Brodie *et al.* (10) and was used to determine FSA and TSA.

Five milliliters of blood is introduced immediately upon withdrawal (from an antecubital vein) into a 90-ml. snap-cap wide-mouth bottle containing 0.5 ml. of 6 *N* HCl[1] and 50 ml. of ethylene dichloride (EDC) containing 1% isoamyl alcohol (v/v). The bottle is capped immediately and shaken vigorously by hand until the blood becomes a brown color. This takes about 30 sec. Thus, less than 1 min. of total time will have elapsed since the hypodermic needle was removed from the blood vessel. The bottle then is shaken on a mechanical shaker for 15 min., after which the EDC extract is separated from the precipitated blood by filtering through dry filter paper (Whatman No. 42).

Twenty milliliters of this EDC extract is transferred to a 90-ml. snap-cap bottle containing 5 ml. of water and 0.25 ml. of a 1% $Fe(NO_3)_3$ solution in 0.070 *N* HNO_3. The bottle is capped and shaken for 10 min. The absorbance of the aqueous layer is measured at 540 mμ, employing the Beckman DU spectrophotometer with 1-cm. cells. The absorbance is converted into FSA by reference to a calibration curve.

A second aliquot (20 ml.) of the EDC extract is shaken for 10 min. with 5 ml. of 0.1 *N* NaOH. Four milliliters of the alkaline aqueous phase is placed in a 50-ml. screw-capped conical flask. The cap is secured, and the closed flask is heated in boiling water for 4 min. The flask then is cooled immediately in cold water, 0.5 ml. of 6 *N* HCl and 30 ml. of EDC are added, and the flask is shaken for 10 min. Twenty milliliters of the separated EDC

[1] This ratio of acid to blood is optimal in maintaining a low blank absorbance.

layer is added to 5 ml. of water and 0.25 ml. of $Fe(NO_3)_3$ reagent in a snap-cap bottle. The bottle is shaken for 10 min., the aqueous layer is removed, centrifuged, and the absorbance is measured at 540 mμ. This is a measure of TSA.

Standard curves are prepared by adding 1 to 50 mcg. of aspirin or SA to freshly drawn blood, then carrying out the described procedure. Regardless of whether aspirin or SA are used, one obtains equivalent calibration curves for TSA.

It is important to use fresh blood when this procedure is followed because the blood blank absorbance increases quickly with time. If fresh blood is incubated for a period of 2 hr. at 37°, an appreciable increase in the usually low blank occurs. Blood which has been stored is useless for the preparation of calibration curves.

Aspirin is calculated as the difference between the FSA and TSA. It is assumed that the SA which appears as a consequence of hydrolysis is derived from aspirin. This seems valid on the basis of the arguments put forth by Leonards (1). These arguments are further reinforced by the fact that it is unlikely that metabolites would partition into organic solvent in the same manner as aspirin.

Serum Salicylate.—Serum salicylate was determined by a slight modification of the method of Routh and Dryer (13). Blood samples were incubated for 2 hr. at 37°. The extruded serum was separated from the coagulum by centrifugation, 2 ml. of serum being used for each analysis.

Ethylene dichloride (EDC) used for the extractions was specially prepared by the Fisher Scientific Co., New York, N. Y. It was accepted by the laboratory on the basis of its low U.V. absorption and its freedom from substances which interfered with the described procedures. All other reagents were reagent grade.

Results.—An experiment was set up to explore the comparative reproducibility of the whole blood method in the determination of TSA and FSA *versus* the Routh and Dryer modification of the method of Brodie *et al.* for total salicylates. Blood samples from 10 subjects were used; on most of these, both 10-min. and 20-min. salicylate levels were determined in duplicate by two different analysts. In the first half of the study, analyst *A* used the whole blood method, while analyst *B* used the serum method. In the second half, the methods were interchanged.

TABLE I.—ANALYST REPRODUCIBILITY IN TERMS OF STANDARD DEVIATION IN MICROGRAMS PER MILLILITER ON BLOOD SAMPLES 10 AND 20 MIN. AFTER ASPIRIN ADMINISTRATION

Analyst	Serum Salicylate	TSA	FSA	Aspirin
A	.14	.67	0	.67
B	.33	.44	.16	.36
Combined	.22	.54	.09	.50

Table I expresses analyst reproducibility in terms of standard deviation (in micrograms per milliliter). The standard deviation for the TSA values is larger than in the case of the FSA, which reflects the more complex procedure in the former determination. In only one case the raw data duplicate readings differed by more than 0.002 o.d. units. This approximates the sensitivity of the instrument. Therefore, the above-described between methods differences are not meaningful.

On all blood samples, serum salicylate, whole blood FSA, and TSA were performed. Determinations were carried out on blood samples drawn immediately before (zero time) and at 10 and 20 min. after the administration of aspirin-containing tablets. The subjects did not fast but had a light breakfast approximately 2 hr. before the experiments. Two 5-gr. tablets of either aspirin[2] or buffered aspirin[3] were administered, followed by 75 ml. of water. Thirty-eight subjects were used with each type of tablet. Most of the subjects have participated in drug absorption experiments for 1 to 3 years; they consisted of males and females of various ages, employed in various capacities in offices, factories, and laboratories. The same group of subjects was used for both preparations.

Analytical data and their standard deviations are presented in Table II. In agreement with the data obtained by Truitt and Morgan (14) and by Sleight (4) for plasma, whole blood salicylate values obtained from persons receiving buffered aspirin are greater than the corresponding values obtained when aspirin was administered. The aspirin/TSA ratio shows that approximately half of the blood salicylate at 10 min. after the ingestion of both types of aspirin tablets is present as the acetylated form. Twenty minutes after the ingestion, the ratio has dropped to approximately 30%.

DISCUSSION

The discrepancies between the present results and those of Leonards (1) are probably because the present results were obtained by stop-time type whole blood analysis, whereas Leonards analyzed plasma with no indicated means of stopping hydrolysis. Leonards (1) obtained about 40% of the total plasma salicylate as acetylsalicylate 10 min. following the ingestion of a solution of sodium acetylsalicylate. On the other hand, he obtained only minimal plasma concentrations of acetylsalicylate following the ingestion of a suspension of insoluble aspirin.

There is a possibility also that aspirin is present at a higher concentration than SA in erythrocytes. Aspirin might be expected to diffuse into the erythrocytes more readily than SA because it is only poorly bound to serum albumin and therefore is present unbound in higher concentration in plasma.

The apparent difference obtained between the serum total salicylate and the whole blood total salicylate is due to the large volume occupied by erythrocytes which contain little salicylate. In the serum assay, the blood is incubated for 2 hr., during which time not only does syneresis occur, but also essentially all of the aspirin present is converted into SA. The SA is then largely concentrated in the serum by virtue of its high affinity for serum albumin. Correction for hematocrit of data in Table II yields comparable TSA values for the two methods. The whole blood salicylate/serum salicylate ratio is, therefore, in the neighborhood of 0.6, which is in line with predictions.

[2] Bayer, lot H1142.
[3] Bufferin. (Randomly selected production tablets.)

TABLE II.—RESULTS OF ADMINISTERING 10 gr. OF ASPIRIN OR 10 gr. OF BUFFERED ASPIRIN TO HUMANS[a]

	Blood or Serum Levels, mcg. of SA/ml.			
	10 min.		20 min.	
	Aspirin	Buffered Aspirin	Aspirin	Buffered Aspirin
TSA $\bar{X}$[b]	3.87	6.79	9.81	15.91
S. D.	3.86	5.23	5.50	6.47
FSA $\bar{X}$[b]	1.93	3.26	6.74	10.71
S. D.	2.52	3.21	4.62	5.10
Aspirin $\bar{X}$[b]	1.93	3.53	3.07	5.20
S. D.	1.91	2.40	1.61	2.07
Serum salicylate $\bar{X}$	6.09	11.30	17.78	27.26
S. D.	5.59	9.36	8.52	12.50
Aspirin/TSA ratio	0.49	0.51	0.31	0.33
Correlation Coefficient Aspirin *vs.* TSA	0.83	0.91	0.65	0.75
TSA/serum salicylate ratio	0.62	0.60	0.55	0.58
Correlation Coefficient TSA *vs.* serum salicylate	0.90	0.96	0.94	0.84
Correlation Coefficient Aspirin *vs.* serum salicylate	0.67	0.67	0.50	0.58

[a] 10 and 20 min. postmedication. [b] Whole blood concentration.

SUMMARY

A reproducible method for the determination of SA and total salicylates is described.

The present work supports the view of Leonards (1) that aspirin is absorbed intact from the gastrointestinal tract. The results differ from those of Leonards in that they demonstrate for commercial tableted aspirin preparations a high proportion of acetylated salicylate in circulating blood following oral ingestion. The discrepancies between the above and the results reported here in the relative amount of acetylated salicylate are considered to be due to the fact that the present determinations were carried out on whole blood, eliminating the complications of hydrolysis during analysis, and the complication of unequal protein-binding of the two forms of salicylate.

Higher levels of acetylsalicylate were obtained for buffered aspirin than unbuffered aspirin. In both cases, half of the salicylate in circulating blood was present in the acetylated form 10 min. after oral ingestion of commercial tablets. Twenty minutes after ingestion, the proportion dropped to 30%.

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