previous findings(21), and the contribution of the bacteria to the matrix, these might well be lipid, or lipid in combination with polysaccharide or protein, as is known to exist in bacterial cell walls(22).

The analytical data given here, together with earlier histochemical studies, lend support to the possibility that a similar mechanism may be involved in formation of calculus, whether it be urinary, submaxillary gland, or supragingival. The contribution of the bacteria is difficult to assess. Supragingival calculus can form in animals in the absence of bacteria(23); bacteria themselves, however, may become calcified(24,25). Apparently, although not essential to the process, the oral bacteria are ordinarily involved.

Summary. Analysis of supragingival salivary calculus disclosed many similarities in composition to urinary and submaxillary gland calculus. All the carbohydrates reported to be present in the latter concretions were found in the supragingival material. In addition the supragingival salivary calculus contained small amounts of sialic acid, xylose and arabinose. From the nature of the carbohydrate components it would appear that both saliva and bacteria contribute to the composition of supragingival salivary calculus.

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Received March 26, 1962. P.S.E.B.M., 1962, v110.

Presence of Acetylsalicylic Acid in Plasma Following Oral Ingestion of Aspirin. (27499)

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Recent investigations have questioned the assumption that aspirin is hydrolyzed in the intestinal tract prior to absorption. Although in several studies aspirin has been noted to be rapidly converted to salicylic acid following its oral ingestion(1,2), the presence of acetylsalicylic acid has been demonstrable in blood for up to an hour(3,4,5). In experi-

TABLE I. Free Salicylate and Acetylsalicylic Acid in Gastrointestinal Tract of Dogs One Hour after Oral Administration of Aspirin.

Dog No.	Wt, kg	Oral aspirin, mg	Amt in GI tract, mg			
			Stomach		Intestine	
			Free	Acetyl	Free	Acetyl
1	12	2400	13	625	10	30
2	8	1600	0	800	0	35
3	10	2000	18	375	5	45

ments employing C^{14} labeled aspirin(4) it was estimated that at least a major portion of the salicylate found in blood is formed by the hydrolysis in the tissues of aspirin absorbed intact.

The present report describes the results of various experiments designed to determine the form of absorption and rate of hydrolysis of aspirin following ingestion. The data to be presented support the hypothesis that aspirin is absorbed intact and that its hydrolysis occurs in the body tissues.

Methods. Five experimental designs were employed in this investigation:

1. The quantities of salicylate and acetylsalicylate were determined in the gastrointestinal washings from rats and dogs on sacrifice at 30 or 60 minutes after oral administration of aspirin.* 2. The concentration of acetylsalicylate and salicylate in the plasma was determined in both dogs and humans following oral ingestion of aspirin and sodium acetylsalicylate. The latter was prepared in solution by neutralizing aspirin with a stoichiometric equivalent of sodium bicarbonate. All preparations were dissolved or suspended in 200 ml water. 3. The concentration of acetylsalicylate and salicylate in plasma was compared following oral ingestion and intravenous injection of sodium acetylsalicylate to dogs. 4. The rate of hydrolysis of acetylsalicylate to salicylate on incubation with canine plasma *in vitro* at 37° was compared with the rate of hydrolysis following its rapid intravenous injection into dogs. 5. Sodium acetylsalicylate was orally administered to unanesthetized dogs bearing hepatic vein and portal vein cannulas(6) and concentrations of free and acetylated salicylate were determined in blood simultaneously withdrawn from both vessels. This is a direct measurement of the effect of the liver on acetylsalicylate.

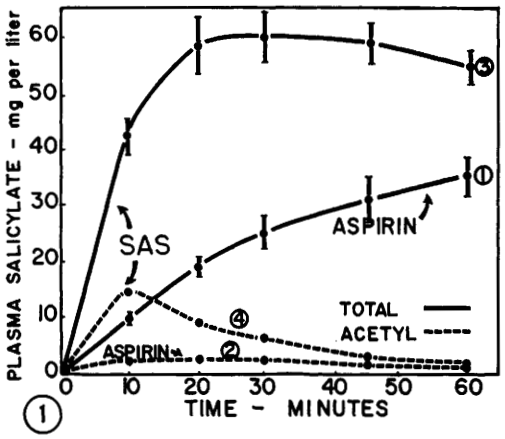
Plasma free salicylate concentrations were determined by the method of Brodie *et al.*(7). This method does not measure acetylsalicylate. Total salicylate was determined by hydrolysis of the plasma with 2 N hydrochloric acid at 100°C for 10 minutes and then using the Brodie method. The acetylsalicylate concentrations were then taken as the difference between total salicylate and free salicylate. It is assumed in these analyses that the material hydrolyzed to salicylate is the acetyl derivative of salicylic acid. This assumption appears justified since it has been shown chromatographically that following the ingestion of aspirin by humans, salicylic acid and aspirin are the only compounds containing the salicyl group demonstrable in plasma by the methods employed(4). In addition, in experiments to be described, no substances hydrolyzable to salicylate were detected in the blood of subjects receiving sodium salicylate orally. Although the presence of salicylate conjugates in plasma has been demonstrated (6) their concentrations were very low, representing less than 1% of the total salicylate.

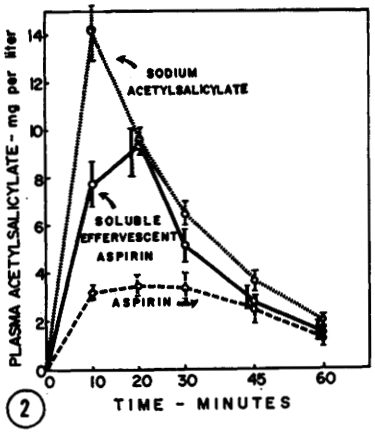
Subjects and dogs were fasted for 12 hours prior to each experiment but water was allowed *ad libitum.*

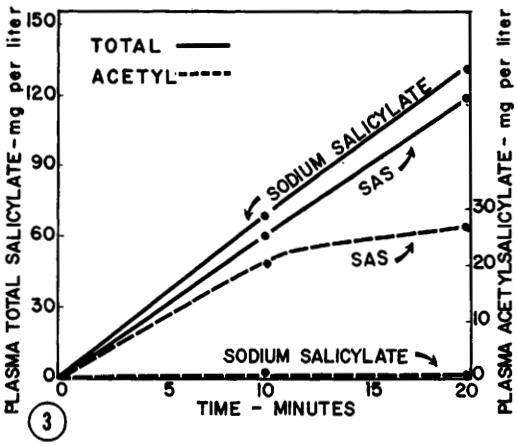
Results. When 3 dogs were sacrificed 1 hour following oral administration of 0.2 g per kg of aspirin (suspended in 200 ml water) only negligible quantities of free salicylate were found in the gastrointestinal washings despite the presence of considerable quantities of intact acetylsalicylic acid (Table I). Similar results were obtained in rats sacrificed 30 and 60 minutes after oral administration of aspirin.

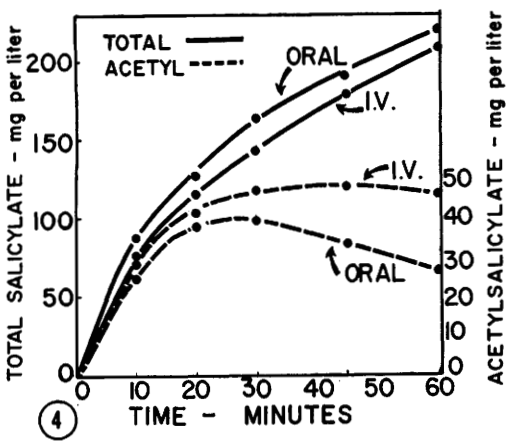
Plasma salicylate and acetylsalicylate concentrations were determined following ingestion of 640 mg of aspirin, or its equivalent as

* Bayer Aspirin having a USPXV disintegration time of less than 10 seconds was used in all experiments.









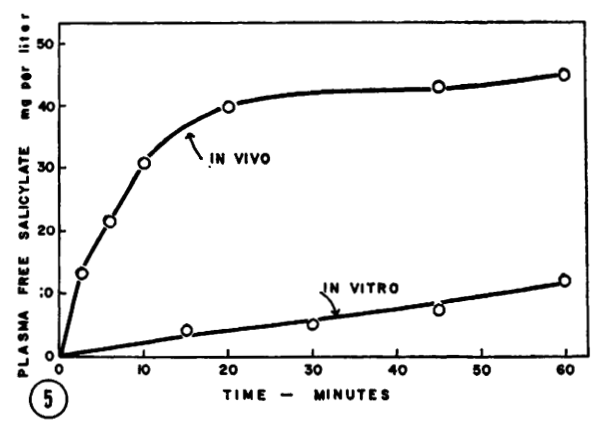


FIG. 1. Average plasma total salicylate (——) and acetylsalicylate (- - - -) levels following oral ingestion of 640 mg of aspirin Curves 1 and 2 or its equivalent as sodium acetylsalicylate (SAS) Curves 3 and 4 by 15 normal human subjects. Results in all figures are expressed as mg of salicylic acid per liter of plasma. Vertical bars represent standard errors.

FIG. 2. Average plasma acetylsalicylate levels in 15 normal human subjects following oral ingestion of 640 mg of aspirin or its equivalent as sodium acetylsalicylate or a commercial soluble effervescent preparation. Vertical bars represent standard errors.

FIG. 3. Plasma total salicylate (——) and acetylsalicylate (- - - -) following the oral ingestion of sodium acetylsalicylate (SAS) and sodium salicylate dissolved in 200 ml of water. Average of 6 normal subjects taking the equivalent of 1920 mg of aspirin.

FIG. 4. Plasma total salicylate and acetylsalicylate following oral administration of 0.2

g per kg of sodium acetylsalicylate compared to that after intravenous injection of the same drug at the rate of 0.15 g per kg per hr. Average results in 10 normal dogs.

FIG. 5. Comparison of rate of appearance of free salicylate from acetylsalicylate in dog plasma incubated *in vitro* to that occurring *in vivo*. Total salicylate levels were comparable in both cases for the entire time period. Average results of 3 normal dogs.

sodium acetylsalicylate (Fig. 1). After ingestion of aspirin suspended in water there was only a slow increase in total plasma salicylate concentration and only minimal quantities of acetylsalicylate appeared in the plasma. In contrast, ingestion of the sodium salt in solution resulted in the rapid appearance of both salicylate and acetylsalicylate in the plasma, the latter constituting after 10 minutes about 40 percent of the total salicylate. After 10 minutes the acetylsalicylate fraction decreased gradually while the total salicylate concentration continued to increase.

Fig. 2 presents in greater magnification the acetylsalicylate plasma concentrations for the 2 aspirin preparations presented in Fig. 1 and for a commercially available soluble effervescent preparation.† The concentration of acetylsalicylate achieved in the plasma is proportional to the increased rate of intestinal absorption of the soluble preparations. These rates have been reported previously(9). The increased levels attained with the soluble preparations are statistically significant ($P < .001$) at 10 and 20 minutes.

The results obtained with ingestion of sodium salicylate compared to sodium acetylsalicylate are presented in Fig. 3. Both preparations were dissolved in 200 ml of water. These are average results of 6 normal human subjects ingesting the equivalent of 1920 mg of aspirin. While similar total plasma salicylate concentrations were attained, only following ingestion of sodium acetylsalicylate was there an appearance in the plasma of a substance assayable as acetylsalicylate by the methods employed.

Fig. 4 illustrates results obtained when sodium acetylsalicylate was infused intravenously into dogs at a rate comparable to that of gastrointestinal absorption, so that similar plasma total salicylate concentrations were achieved. The acetylsalicylate concentrations were also similar for the first 30 minutes.

† Alka Seltzer - Miles Products Co. This is the only readily available commercial aspirin preparation which is dissolved in water prior to ingestion.

Fig. 5 compares the rate of appearance of free salicylate on incubation of sodium acetylsalicylate in canine plasma *in vitro* with that following a single intravenous injection of the drug to dogs. While initial plasma total salicylate concentrations were the same (not illustrated) there was only a slow hydrolysis of the acetylsalicylate *in vitro*, while *in vivo* there was rapid hydrolysis as evidenced by the rapid increase in free salicylate.

When aspirin was ingested by 3 dogs bearing hepatic and portal vein cannulas, acetylsalicylate concentrations in the portal vein were higher than those in the hepatic vein while free salicylate concentrations were higher in the hepatic than the portal vein (Table II).

Discussion. The essential absence of free salicylate in the gastrointestinal tract during aspirin absorption is further evidence for the intact absorption of aspirin. The observed appearance of acetylsalicylate in the plasma following its oral ingestion confirms the observations of others(3,4,5). However, much higher levels were obtained in the present experiments. It has been noted(9) that soluble preparations of aspirin are more rapidly absorbed by the gastrointestinal tract than relatively insoluble preparations, as measured by the appearance of a salicylate in the plasma. The results reported here demonstrate the attainment of greater concentrations of acetylsalicylate following ingestion of dissolved,

TABLE II. Free Salicylate and Acetylsalicylate in Portal and Hepatic Veins of the Dog after Oral Administration of 0.2 g per kg of Sodium Acetylsalicylate.

Min. after oral dose	Plasma salicylate concentration			
	Free salicylate		Acetylsalicylate	
	Portal	Hepatic	Portal	Hepatic
10	16	27	27	10
20	51	60	52	15
30	88	104	67	29
40	115	130	70	30
50	135	150	77	35
60	152	165	93	48

Results expressed as mg of salicylic acid/l of plasma and are averages of 3 dogs.

rather than an insoluble, aspirin preparation even though the latter was given in the form of rapidly disintegrating tablets. The possible analgesic superiority of the soluble preparations when compared to ordinary aspirin may be attributable to these concentration differentials.

The rational of using sodium acetylsalicylate may be questioned on the grounds that the free HCl in the stomach would react with it to liberate acetylsalicylic acid. However, it was found experimentally that with the volumes of solutions that were used, the latter did not precipitate but remained in supersaturated solution.

The experiments comparing the consequences of oral *vs* intravenous administration of sodium acetylsalicylate also provide evidence that the intervening processes during the passage of aspirin into the plasma from the gut do not result in any significant hydrolysis of acetylsalicylate, since the acetylsalicylate concentrations attained are similar whether aspirin is administered intravenously or orally (Fig. 4). The lower concentration of acetylsalicylate on oral administration after 30 minutes is perhaps due to the presentation by the latter route of the aspirin to the liver via the portal vein prior to its entrance into the general circulation. Results of the experiments in dogs with catheters in the portal and hepatic veins (Table II) demonstrate the contribution of the liver to hydrolysis of acetylsalicylate.

Therefore, it seems likely that aspirin on oral ingestion is largely absorbed intact into the plasma and that hydrolysis proceeds rapidly following this absorption. That tissue rather than plasma processes are the prime factors in this hydrolysis is supported by the slow rate of hydrolysis by plasma *in vitro* and the rapid rate *in vivo,* and by the demonstrated net uptake of acetylsalicylate by the liver and net output of free salicylate. Evidence for hydrolysis of acetylsalicylic acid by liver and kidney has been previously reported (10).

Summary. The absorption and hydrolysis of aspirin has been examined under various experimental conditions. Aspirin appears to be absorbed intact from the gastrointestinal tract. High concentrations of acetylsalicylate in the plasma can be demonstrated following oral ingestion of soluble but not insoluble aspirin preparations. This is correlated with the increased rate of absorption of the soluble products. There appears to be a rapid hydrolysis of acetylsalicylate *in vivo* and it is suggested that the principal site of this hydrolysis is the tissues. Direct evidence for hydrolysis of acetylsalicylate to salicylate by the liver of the dog has been demonstrated.

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Received December 18, 1961. P.S.E.B.M., 1962, v110.