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Studies on the Hydrolysis of Acetyl-1-C^{14}-Salicylic Acid*

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In recent years, studies of the metabolism of salicylates have been concerned primarily with the concentration of salicylates and metabolities in various body fluids. The absorption of salicylates and hydrolysis of acetylsalicylic acid have received little attention. The presence of conjugated forms of salicylates in plasma is still in dispute. Brodie, Udenfriend and Coburn (1) were unable to demonstrate the presence of appreciable quantities of salicyluric acid in the plasma, although Kapp and Coburn (2) had shown it to be one of the main conjugated forms in the urine. Lester, Lollie and Greenberg (3) reported that in a 75 Kg. person, 27 per cent of the salicylate in the plasma existed in a conjugated form thirty minutes after ingestion of a 0.65 gm. dose of acetylsalicylic acid. They measured the difference in salicylate levels before and after hydrolysis and assumed the conjugated form was acetylsalicylic acid. Smith and co-workers (4) found no free acetylsalicylic acid in the plasma of men or dogs one hour after intravenous administration or at any time after oral administration.

One of the outstanding difficulties in previous investigations has been the lack of a method for the identification of unhydrolyzed aspirin in a body fluid or tissue. In an attempt to solve this problem, we synthesized acetylsalicylic acid labelled with C^{14} in the acetyl group (5). Hydrolysis of this radioactive aspirin would produce salicylic acid and an acetate group containing C^{14}. If unhydrolyzed radioactive aspirin were present in a fluid or tissue, it could be detected by a combination of radioactivity measurements and chemical techniques.

Experimental

Before attempting to study in vivo hydrolysis, the conditions for the hydrolysis of aspirin in vitro were investigated. Varying amounts of aspirin were added to plasma samples and hydrolysis was performed with or without the addition of hydrochloric acid. Values shown in Table I indicate a low rate of hydrolysis at 37° C. without the addition of hydrochloric acid. Complete hydrolysis occurred within 10 hours at 37° C. or 3 hours at 60° in the presence of hydrochloric acid (0.5 ml. of 6N HCl to 1 ml. of

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plasma). The results of these experiments indicated that at room temperature the hydrochloric acid-ethylene chloride mixture commonly used to extract salicylate from plasma, would not cause appreciable hydrolysis of any aspirin which might be present.

Table I

Hydrolysis of Aspirin in Vitro.

Time in Minutes	Aspirin + Plasma	Aspirin +	plasma +		HCI
	200 μg./ml. at 37° C. per cent	100 μg./ml. at 37° C. per cent	200 μg./ml. at 55° C. per cent	200 μg./ml. at 60° C. per cent	300 μg./ml. at 60° C. per cent.
2	3	100%	7	4	2
30	7	in	46	65	59
60	14	10 hours	68	86	84
90	28		84	93	96
120	28		84	96	96
150			88	100	98
180					100

To study the rate of in vivo hydrolysis, rabbits were given radioactive aspirin (specific activity 2990 counts/minute/mg.) both intravenously and orally. During the experiments the rabbits were kept in a closed respiratory system to facilitate collection of the expired carbon dioxide. The CO_2 was bubbled through traps containing a solution of barium hydroxide. A small dose (80mg./kg.) of acetyl-1-C^{14}-salicylic acid was administered intravenously and the amount of free salicylate was determined immediately after withdrawal of blood samples and also after complete acid hydrolysis by the method of Brodie et al (1). Complete hydrolysis was assured by heating 1 ml. of plasma and 0.5 ml. of 6N HCl for at least 3 hours at 60° C. The results are shown in Table II. It appears that practically all of the aspirin was hydrolyzed within 30 minutes. Plasma samples were extracted with ethylene chloride and the extracts chromatographed using a solvent of butanol saturated with water and 2 per cent by volume of glacial acetic acid. It had previously been determined that aspirin has an R_f value of 0.84 and salicylic acid of 1.0 with this solvent. The radioactivity of the various areas on the chromatographic strip was determined by cutting uniform segments (5 mm. wide) of the entire chromatogram and counting their activity with a 1.4 mg./cm.^{2} mica window counter at a distance of 6 mm. All of the radioactivity was found in the aspirin band, although the activity was only slightly above the background count. Another rabbit was given a larger dose (400 mg./kg.) of the radioactive aspirin intravenously, and the rate of hydrolysis followed as before. The results in Table III show

a slower rate of hydrolysis than with the smaller dose, but this difference could reasonably be attributed to the difference in doses. Plasma samples (3 ml.) were extracted with ethylene chloride and found to contain appreciable radioactivity. The activity on the original spot on the paper strips and in the band with an R_f of 0.84 is also shown in Table III. Since virtually all of the activity present in the plasma migrated with the aspirin, it appears that all of the measurable activity in the plasma is due to the unhydrolyzed, labelled aspirin. Aliquots of the $BaCO_3$ collected in the CO_2 traps were plated on aluminum planchets and counted under the thin window Geiger tube. It was found that the rabbit excreted only 0.4 per cent of the total radioactivity as carbon dioxide within the 107-minute period.

Table II

Hydrolysis of Aspirin After the Intravenous Administration of 80 mg./kg. of Radioactive Aspirin

Time After Administration min.	Salicylate Level Before Hydrolysis μg./ml.	Salicylate Level After Hydrolysis μg./ml.	Hydrolysis of Aspirin per cent
10	196	260	75.4
22	204	218	93.6
33	195	204	95.6
47	182	186	97.8
82	130	132	98.5

Table III

Hydrolysis of Aspirin After the Intravenous Administration of 400 mg./kg. of Radioactive Aspirin

Time After Administration min.	Salicylate Level Before Hydrolysis μg./ml.	Salicylate Level After Hydrolysis μg./ml.	Hydrolysis of Aspirin per cent	Radioactivity Original Spot cts/min.	Radioactivity Aspirin Spot R_f 0.84 cts/min.
4	416	1084	38.4	2459	2475
13	554	848	65.3	989	1051
27	580	674	86.1	236	254
43	520	578	90.0	30	33
72	450	482	93.5	26	22
107	430	436	98.6	20	18

To determine whether the activity of radioactive acetate would parallel that of radioactive aspirin after intravenous administration, a rabbit was given 60 mg. of the original radioactive sodium acetate-1-C^{14} used in the synthesis of acetyl-1-C^{14}-salicylic acid. Plasma samples withdrawn at various time intervals were lyophilized and plated on aluminum planchets and counted as described earlier. Within 6 minutes, the activity of the plasma had dropped practically to zero indicating that the radioactive acetate radical disappeared very rapidly from the plasma after being split from the salicylic acid radical.

In an attempt to obtain information concerning the hydrolysis following oral administration of aspirin, a rabbit received a 460 mg./kg. dose of radioactive aspirin orally. Unhydrolyzed aspirin appeared in the plasma in approximately ½ hour and persisted for about 2 hours (Table IV). Ethylene chloride extracts of the first four plasma samples exhibited radioactivity. When chromatographed, the activity was found in the characteristic band for aspirin with an R_f of 0.84. The activity of these bands was equivalent to that measured on the original solute spots of the chromatograms.

Table IV

Hydrolysis of Aspirin After Oral Administration of 460 mg./kg. of Radioactive Aspirin

Time	Salicylate Level		Hydrolysis of Aspirin	Radioactivity	
After Administration	Before Hydrolysis	After Hydrolysis		Original Spot	Aspirin Spot
min.	μg./ml.	μg./ml.	per cent	cts/min.	cts/min.
22	334	330	100	41	43
46	379	392	96.6	107	118
73	368	407	90.3	20	31
109	395	405	97.5	16	21
150	430	426	100	....	....

In another similar experiment, a rabbit received 590 mg./kg. of radioactive aspirin orally. Again the unhydrolyzed salicylate appeared in the plasma and remained until the end of the 2-hour experiment. The activity of the original spot was all found in the aspirin band after development of the chromatograms. In this experiment, the urine was also collected by catheterization and analyzed for radioactivity. It was found that 0.1 per cent of the original dose was present in the urine. When a sample of the urine was chromatographed, all of the activity (426 counts per min. on the original spot and 460 counts per min. on the aspirin band) was found in the band with an R_f of 0.85.

Discussion

The data from the in vitro hydrolysis of aspirin suggests that even in the presence of hydrochloric acid, the hydrolysis proceeds slowly at 37° C. These findings are in direct contrast to the rapid hydrolysis achieved by the tissues and enzyme systems in the blood.

The major difficulty in previous studies of the rate of in vivo hydrolysis of aspirin has been the lack of a method for the identification of unhydrolyzed aspirin in the blood or urine. The use of acetyl-1-C^{14}-salicylic acid coupled with measurements of radioactivity and chromatography helped solve this difficulty. The hydrolysis of aspirin following intravenous injection can be followed readily by the application of these methods. It is reasonable to assume that less unhydrolyzed aspirin would be present in the plasma after oral than after intravenous administration since intestinal hydrolysis and absorption would decrease the amount of aspirin introduced into the blood stream. Nevertheless, appreciable quantities of unhydrolyzed aspirin are found in the plasma and urine after oral administration.

SUMMARY

1. The in vitro and in vivo hydrolysis of aspirin have been studied. When radioactive aspirin was injected intravenously into a rabbit, 90 per cent or more of it was hydrolyzed within 30 to 40 minutes.
2. After the oral administration to a rabbit, unhydrolyzed aspirin was found in the plasma within ½ hour and persisted for about 2 hours.

References

(1) Brodie, B. B., Udenfriend, S. and Coburn, A. F., J. Pharmacol. Exptl. Therap., *80*, 114 (1944)
(2) Kapp, E. M. and Coburn, A. F., J. Biol. Chem., *145*, 549 (1942)
(3) Lester, D., Lolli, G. and Greenberg, L. A., J. Pharmacol. Exptl. Therap., *87*, 329 (1946)
(4) Smith, P. K., Gleason, H. L., Stoll, C. B. and Orgorzalek, S., J. Pharmacol. Exptl. Therap., *87*, 237 (1946)
(5) Clappison, J. W., Hummel, J. P., Paul, W. D. and Routh, J. I., J. Am. Pharm. Assoc., *40*, 532 (1951)

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