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The Distribution of C^{14} Labelled Salicylates in Rat Tissues*

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During the period of development of salicylate therapy, it was demonstrated that many body membranes were permeable to salicylates, and that appreciable quantities were excreted in the urine. With the development of a satisfactory quantitative method by Brodie *et al.* (1) the salicylate concentration in body fluids and urine could be determined. More recently the successful labelling of salicylates with C^{14} has placed investigation of salicylate distribution in animal tissues on a more practical basis. The carboxyl group of salicylic acid (2) (3) (4) and the acetyl group of acetylsalicylic acid (5) have been tagged with radioactive carbon. These labelled compounds have been employed in experiments on the metabolism and distribution of the salicylates (3) (4) (6). The most complete distribution study was performed by Schayer (3) after the administration of C^{14} carboxyl labelled salicylic acid to rats. He determined the radioactivity in various organs and the excretory products 24 hours after administration.

Since Schayer found that practically all of the administered salicylate was excreted in 24 hours, it was thought that a shorter period should be employed in such studies. Preliminary experiments indicated that a three hour period would be satisfactory. The following study was undertaken to compare the distribution of carboxyl labelled salicylic acid and acetyl labelled acetylsalicylic acid in body fluids and tissues of rats.

Experimental

Carboxyl labelled salicylic acid was synthesized from C^{14} carbon dioxide and sodium phenolate by a modification of the method of Mandel and Smith (2). The final product, twice recrystallized, with an average yield of 32 per cent, had a specific activity of 318,400 counts per minute per milligram when counted in a gas-flow, windowless counter.

Acetyl labelled acetylsalicylic acid was synthesized by a method developed in our laboratory (5). Sodium acetate-1-C^{14} was converted to acetyl chloride by reaction with benzoyl chloride; then the radioactive acetyl chloride was allowed to react with salicylic acid in the presence of dimethylaniline. After recrystallization of the final product, a 43.2 per cent yield of the acetyl labelled compound, with a specific activity of 196,100 counts per minute per milligram, was obtained.

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Seven male rats were given the radioactive salicylates. Three received carboxyl labelled salicylic acid by stomach tube, whereas two rats received it by intraperitoneal injection. The acetyl labelled compound was fed to one rat by stomach tube, another received it intraperitoneally. Each animal was given radioactive salicylate equivalent to 3,184,000 counts per minute diluted with 100 mg. of non-radioactive material. A small quantity of sodium bicarbonate was added to facilitate solution in a volume of 2 ml. All animals were placed in a vacuum desiccator fitted with a respirator that permitted collection of expired carbon dioxide in a sodium hydroxide solution. After a period of 3 hours, all animals were anesthetized to facilitate drawing blood from the heart. Specimens of kidney, brain, spleen, lung, heart, liver, intestine and skeletal muscle were then removed. The urine in the bladder was added to that collected during the 3 hour period.

Immediately upon removal, the tissues were frozen with dry ice. Subsequently they were pulverized with dry ice and lyophilized. Small portions of the dried tissues were homogenized with ether and plated on aluminum planchets. Aliquots of the sodium hydroxide solution containing the expired carbon dioxide precipitated as barium carbonate were also plated for counting. Urine radioactivity was determined in a similar fashion. A gas-flow windowless counter was used to measure the radioactivity of the specimens.

The specific activity of each tissue was calculated, after applying a correction factor for the weight of the protein layer. The activity of each organ or tissue based on its estimated total dry weight, and the radioactivities excreted in the urine and expired carbon dioxide were determined. The results for each animal were tabulated and the total recovery of radioactivity from the various tissues approximated. Table I presents typical results after the administration of carboxyl C^{14} labelled salicylic acid. Specific activities and percentage recovery of administered radioactivity for each rat is shown in Table II.

As indicated by the data, radioactivity was found in every tissue and fluid tested, but varied considerably from animal to animal. The results substantiate earlier work in that most tissues of the body are readily permeable to salicylates which are rapidly excreted. The total radioactivity excreted in the urine exhibited considerable variation. This was probably caused by the short period of urine formation (3 hours) and the difficulties of quantitative collection. In general, as seen in Table III, larger quantities of labelled salicylates are associated with the larger urine volumes. The carbon dioxide excretion also varied with each animal but the percentage of the total radioactivity was higher in rats administered the acetyl labelled aspirin (Table III).

Discussion

The radioactivity found in every organ and tissue examined confirmed the choice of a three hour period for distribution studies. Both types of labelled salicylates when administered orally or intraperitoneally were found in highest quantity in the skeletal muscle, intestine, blood and liver. Apparently within a 24 hour period, as observed by Schayer (3), the animal is able to absorb, distribute, and almost completely excrete the salicylates in the urine.

Experimental difficulties in the quantitative collection of urine add to the problem of interpreting the results shown in Table III. When a volume of 5 cc. or more was collected, the data indicate that the rat excreted more radioactivity after carboxyl labelled salicylic acid than after the acetyl labelled compound. Similar results were obtained in other studies using rabbits as the experimental animal (7). In these experiments, the labelled salicylates were administered intravenously and the urine was collected with an indwelling catheter.

As expected, the excretion of radioactivity in the expired carbon dioxide was greater in animals receiving acetyl labelled aspirin than those receiving the more stable carboxyl labelled compound (Table III). In the rabbit experiments (7) mentioned above, the same trend in carbon dioxide excretion of radioactivity was observed. Several investigators (3) (4) (6) have indicated that the excretion of radioactivity in the carbon dioxide after the administration of carboxyl labelled salicylic acid is negligible. Schayer (3) reported 0.2 per cent of the total administered radioactivity excreted in the carbon dioxide in 24 hours. As may be seen in Table III from 0.05 to 4.0 per cent of the total was excreted in the carbon dioxide during the 3 hour period. Similar values were obtained in experiments with rabbits. These results indicate a small but appreciable decarboxylation of the salicylic acid molecule by the rat and rabbit.

The values for percentage recovery listed in Table II help account for the variation in the tissues. When both the excretion and recovery were low, the material was apparently being absorbed more slowly in that particular animal with resultant low specific activities in the organs. The animals given acetylsalicylic acid displayed relatively low specific activities in their tissues with increased excretion, particularly in the expired carbon dioxide. There was no correlation between the percentage recovery and the route of administration of the salicylates. They seem to be absorbed as well from the peritoneal cavity as from the intestinal tract. The variations are probably due to differences in the individual animals.

It is possible that a portion of the radioactivity in the tissues and urine following the administration of the acetyl labelled aspirin may be represented by acetyl groups split off the compound. In a study of the hydrolysis of acetyl labelled aspirin (8) rabbits were

Table I

Distribution of Carboxyl C^{14} Salicylic Acid in the Tissues of a 250 gm. Rat

Organ	Percentage of total body weight	Wet wt. of Organ gm.	Percentage of Water	Dry wt. of Organ mg.	Specific Activity cts/min./mg.	Total counts per min.
Kidneys	0.83	2.08	81	390	33	12,870
Brain	0.72	1.80	76	430	23	9,890
Intestine	4.60	11.50	75	2880	33	95,040
Skeletal Muscle	20.00	50.00	75	12500	14	175,000
Spleen	0.26	0.65	77	150	20	5,000
Blood	5.80	14.50	55	6520	47	306,440
Lungs	0.56	1.40	78	321	65	20,150
Heart	0.37	0.93	79	200	54	10,800
Liver	4.66	11.65	75	2913	31	89,900
Urine						61,760
Expired CO_2						5,800
Recovery						792,650 (25%)

Table II

Distribution of Radioactive Salicylate in Rat Tissue.

	Carboxyl-labelled Salicylic Acid					Acetyl-labelled Aspirin	
	By Stomach Tube			Intraperitoneally		Orally	Intraperitoneally
Animal No.	1	2	3	4	5	6	7
Weight	268 gm.	250 gm.	314 gm.	326 gm.	292 gm.	344 gm.	292 gm.
Kidneys	71*	33	13	56	11	44	24
Brain	129	23	4	27	7	12	5
Intestine	552	33	7	79	12	24	31
Muscle	73	14	6	21	5	9	7
Spleen	89	20	7	36	5	20	38
Blood	50	47	16	62	19	21	18
Lungs	29	65	17	40	10	22	18
Heart	86	54	9	49	12	14	17
Liver	38	31	8	37	10	14	16
Urine (total)	110	61,760	7,448	972	88,680	31,190	32,424
Expired CO_2 (total)	76,130	5,800	1,532	8,720	137,400	592,000	1,733,300
Recovery (total)	3,319,250	792,650	305,986	1,340,432	534,967	1,142,660	2,179,548
Per cent Recovery	100	25	10	42	17	36	69

*All results for individual tissues are expressed as specific activities in counts/min./mg.

Table III

Excretion of Radioactivity in the Urine and Expired Carbon Dioxide.

	Carboxyl Labelled Salicylic Acid					Acetyl Labelled Aspirin	
Rat No.	1	2	3	4	5	6	7
Weight	268 gm.	250 gm.	314 gm.	326 gm.	292 gm.	344 gm.	292 gm.
				Urine			
Volume	2 drops*	20 cc.	1 cc.	2 cc.	20 cc.	5 cc.	21 cc.
Total counts/min.	110	61,760	7,448	806	88,680	31,190	32,424
				Carbon Dioxide			
Total counts/min.	76,130	5,800	1,532	8,720	137,400	592,000	1,733,300
Per Cent of Total Administered	2.0	0.2	0.05	0.3	4.0	19.0	54.0

*Urine lost during anesthesia

injected intravenously with C^{14} labelled acetate. Within 6 minutes the radioactivity had disappeared from the blood. These results indicate a rapid utilization of any acetate groups split from the acetylsalicylic acid.

SUMMARY

1. The carboxyl group of salicylic acid and the acetyl group of acetylsalicylic acid were labelled with C^{14}. These compounds were administered orally and intraperitoneally to rats. After 3 hours the specific activites of various organs and tissues, and the total radioactivity excreted in the urine and expired carbon dioxide were determined.

2. Every tissue examined contained radioactivity with the highest activities in the skeletal muscle, intestine, blood and liver. The activity in the expired carbon dioxide was greatest after the administration of acetyl labelled acetylsalicylic acid, whereas in the urine more radioactivity resulted from the administration of the carboxyl labelled compound.

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