SHORT COMMUNICATION

THE USE OF COMBUSTION TECHNIQUE OF TISSUE PROCESSING FOR THE STUDY OF THE DISTRIBUTION OF ^{14}C-SALICYLATE IN RATS AFTER ORAL ADMINISTRATION

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Summary : The distribution of ^{14}C-salicylate (150 *mg/kg*, orally) in rat tissues was studied, at different time intervals upto 2 hr. A fairly rapid distribution was noted and a peak level was found to occur at 30 min after administration of the labelled drug in all the tissues studied.

The combustion technique was used for tissue processing to determine the 'radioactivity as compared to the digestion method of tissue processing utilised by Sturman *et al.* (5) for their studies. The combustion technique was found to be more sensitive, as at a dose of 150 *mg/kg* (0.5 *ml* containing 5 uCi) administered orally, there was a greater level of radioactivity in all tissues as compared to the levels found by Sturman *et al.* (5) for a dose of 200 *mg/kg* (0.2 *ml* containing 2 uCi) administered intraperitoneally.

Key words : ^{14}C labelled salicylate tissue processing

INTRODUCTION

The distribution of salicylate in different tissues has been studied since 1946. Sturman *et al.* (5) used the method of digestion of the tissues in studying the distribution of ^{14}C-salicylate in the mouse but, lately oxidation equipment are being used in tissue processing which utilise the combustion principle based on the original Pregl-Roth procedure (4).

It has been shown by Hunt and Gilbert (2) who conducted a comparative study of digestion and combustion techniques for a number of tissues, that combustion procedures produced consistently high recoveries. It was therefore decided that the distribution of ^{14}C-salicylate in different tissues should be studied using the combustion technique so that a comparison could be made with the digestion method used by Sturman *et al.* (5).

Also to enable to find the distribution of ^{14}C-salicylate in different areas of the gastrointestinal tract, the oral route of administration was chosen for the present study while Wolff *et al.* (6) and Sturman *et al.* (5) used the intraperitoneal route for their studies.

MATERIALS AND METHODS

Adult Male Wistar rats weighing between 205 and 238.5 *g* were used for the study. A dose of 150 *mg/kg* of ^{14}C-salicylate (0.5 *ml* containing 5 uCi) was administered to fasted rats (for 24 hr prior to commencement of the experiment) orally by stomach tube. The animals were anaesthetized and killed at 15, 30, and 120 min post injection. Some tissues (kidney, lung, heart, spleen, liver, fat, muscle and brain) were isolated, immediately cleaned in physilogical saline, blotted on filter paper and part of the tissue was cut, weighed on a torsion balance, and immediately placed on tin foil resting on dry ice. This freezing process was necessary to prevent any change in chemical state occuring in the tissue due to enzyme activity.

Before the rats were killed, blood was extracted by intracardiac puncture and a definite volume was directly used for radioactivity measurement.

The tissues were than heated in a biological oxidizer (Harvey biological oxidizer), to a temperature of 800°C in a steam of oxygen. The ^{14}C-labelled isotope comes off as $^{14}CO_2$ which is trapped in 10 *ml* of scintillation fluid and is then counted for radioactivity. Besides the above tissues, stomach, small intestines and large intestines, were also studied for ^{14}C-salicylate distribution. The method adopted for study of radioactivity from these tissues was the solubilization method. The intestines and the stomach were cleaned of their contents, immediately after removal, by passing saline through them using a syringe. The tissues were then blotted on a filter paper, weighed and immediately taken in 2-n-methanolic potassium hydroxide (1 *ml*/50 *mg* of tissue). This was followed by heating the tissues in preheated water bath at 60°C for 2 hr. This process was done to homogenize the tissues. The flasks were agitated from time to time and at the end of 2 hr the homogenates were made up to volume with methanolic potassium hydroxide, mixed and left for 5-15 min to precipitate out all solid matter Then 0.1 *ml* aliquots of each digest supernatant was pipetted into separate scintillation vials. To this, 10 *ml* of scintillation fluid was added and used for counting radioactivity.

Radioactivity measurement :

Radioactivity was measured using a liquid scintillation spectrometer (Packard Instruments Company) with a tracer lab Coru/matic 100 dual channel spectrophotometer.

Two vials were set up as blanks with only scintillation fluid. Four other vials were set up as internal standards with 0.1 *ml* of ^{14}C-salicylate, to which 10 *ml* of scintillation fluid was added. Also to one vial of each batch of tissue digests, 0.1 *ml* of internal standard solution was added to correct for quenching in each tissue digest.

The scintillation fluid (counting solution) consisted of; 2–ethoxy ethanol 300 *ml*, formic acid 10 *ml*, toluene 700 *ml*, 1, 4-bis-(5-phenyloxazol-2-yl) benzene (POPOP) 0.1 *g* and 2, 5-diphenyloxazole (PPO) 4.0 *g*.

The radioactivity in counts/min was finally converted to *µg/g* or *µg/ml* (in the case of blood) for the tissue samples. (All chemicals were obtained from Sigma Chemical Co., London).

RESULTS

The determinations of radioactivity from different tissues are shown in Table I.

TABLE I : Distribution of ^{14}C-salicylate expressed as *µg/g*, in tissues of the rat after administration of a dose of 150 *mg/kg*, orally.

Tissue	*Time (min) after administration*			*Data of Sturman et al.* (5)* *at 15 min*
	15	30	120	
Kidney	2304.49	8802.66	2019.86	90.00
Liver	500.69	783.09	258.84	120.00
Blood**	604.50	976.07	427.93	167.00
Spleen	143.14	321.33	145.26	63.00
Lung	356.81	534.48	193.62	
Heart	318.32	374.69	135.88	80.00
Fat	44.85	84.11	115.62	
Brain	50.35	119.63	25.78	48.00
Stomach		2750.10	1035.00	
Small intestines		218.00	176.00	
Large intestines		25.10	24.80	

Values represent mean from 5 animals

*A dose of 200 *mg/kg* was used by Sturman *et al.* (5).

**The values for this tissue are expressed as *µg/ml* of ^{14}C-salicylate.

A fairly rapid distribution of the salicylate was noted in the tissues studied. The peak distribution in all the tissues, except the fat occurred at 30 min after administration. After 30 min, all tissues, except the fat showed a fall in the level of radioactivity.

A comparison of the present results was made with the results shown by Sturman *et al.* (5) for the 15 min time interval which is shown in Table I.

DISCUSSION

The pattern of distribution of ^{14}C-salicylate observed during the present study is similar to that reported by Sturman *et al.* (5). A peak level in all the tissues was observed at 30 min after administration. However, a comparison with the results of Sturman *et al.* (5) of the amounts present in different tissues at the 15 min time interval (See Table I) showed that the amount of radioactivity found in the tissues at a dose of 150 *mg/kg* (0.5 *ml* containing 5 uCi) from the present study was greater than that shown by Sturman *et al.* (5), for a dose of 200 *mg/kg* (0.2 *ml* containing 2 uCi). The difference in the results may be attributed to the different methods used and it has also been shown that combustion technique produces higher recoveries from tissue samples as compared to digestion technique (2). In view of the preliminary data presented in this paper, it may be pointed out that, tissue distribution may have to take into account the type of tissue processing being adopted. Also, systematic studies would be required to establish the tissue distribution of ^{14}C-salicylate by the combustion method of tissue processing.

It should however, be noted that the difference in the results may also be due to the fact that, in the combustion technique only a portion of the tissue is required to be weighed and used for radioactivity measurement while, for a truly representative sample it is necessary to homogenise the whole tissue and remove an aliquot of the homogenate for processing and counting. Rats were used for the present study while Sturman *et al.* (5) used mice for their studies and therefore, species difference may also offer a possible explanation for the apparent disagreement as it has been shown that variations in drug binding to plasma protein among species may play a role in determining differences in tissue levels of drug (1) and such a variation with salicylate has actually been reported (3).

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