Effects of Iodinated Salicylates on Metabolism of Rats and Mice.* (27414)

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Although numerous derivatives of salicylic acid have been studied for their effects on oxygen consumption in man and animals(1-7) no reports have been found on the effects of halogenated salicylates in this respect. Therefore, it was decided to determine the effects of various halogenated salicylates on oxygen consumption of rats. The following halogenated salicylates were used: 5-monoiodo - 2 - hydroxybenzoate, 5 - monobromo - 2-hydroxybenzoate, 3,5-diiodo-2-hydroxybenzoate, 5 - monoiodo - 3 - hydroxybenzoate and 4-monoiodo-2-hydroxybenzoate. Sodium salicylate and 3-hydroxybenzoate were used for reference experiments. The majority of these compounds were tested with rats under 3 conditions; normal, thyroidectomized and hypermetabolic. Also, the effects of sodium salicylate and 5-monoiodo-2-hydroxybenzoate were studied in mice. The results have been published in part(8).

Materials and methods. The 5-iodo-2-hydroxybenzoic acid, 5-bromo-2-hydroxybenzoic acid and 5-iodo-3-hydroxybenzoic acid were made especially for these experiments by Cyclo Chemical Corp. The 3,5-diiodo-2-hydroxybenzoic acid was obtained from Eastman Organic Chemicals. 3-Hydroxybenzoic acid was obtained from K and K Laboratories. Dr. R. Michel kindly supplied 4-iodo-2-hydroxybenzoic acid(9). The sodium salicylate was Fisher Certified Reagent.

Compounds in the form of the sodium salt were dissolved in 10 ml of 0.02 N NaOH and then made up to 100 ml with water. If the compound was in the acid form, an equivalent amount of 1.0 N sodium hydroxide was added to neutralize the acid, then 10 ml of 0.02 N NaOH was added and the volume made up to 100 ml with water. Previous experiments with this amount of sodium hydroxide have demonstrated no effect on metabolism. Also, the many negative experiments in this and previous work with compounds dissolved in this manner indicated that this solvent does not change the metabolic rate. The solutions were injected intraperitoneally. The normal rats and the rats made hypermetabolic were obtained from Carworth Farms. Thyroidectomized rats were obtained from Charles River Breeding Laboratories. The hypermetabolic rats had received 20 μg of 3,3',5-L-triiodothyronine in their drinking water for 2 months. This dosage was increased to 32 μg 2 weeks before the experiments. The thyroidectomized rats were used 5 months after surgical thyroid removal. These animals were kept on an iodine low diet (General Biochemicals) and had ceased to gain weight at time of experiment. The white mice were obtained from Carworth Farms. Oxygen consumption of the rats was determined by a closed system(10) and oxygen consumption of the mice was determined by a Servo Spirometer (Custom Engineering and Development Co.). Oxygen consumptions were converted into metabolic rates by the usual methods. In each series, a number of animals not receiving any of the compounds were studied for controls. Food was withdrawn from the rats the night before experiment but not from the mice.

Results. The results are given in Tables I to III. Table I shows the results with the compounds which increased metabolism. When dosages from 12.8 mg to 30.0 mg of sodium salicylate per 100 g body weight were used, there were increments in metabolism both in normal and hypermetabolic rats. In thyroidectomized animals there was no increase in metabolism. In fact, at the higher dose levels there was a fall in metabolism. In contrast, when dosages from 8.4 mg to 20.6 mg per 100 g of 5-iodo-2-hydroxybenzoate were used, there was an increment in oxygen consumption in thyroidectomized rats as well as in normal and hypermetabolic animals. Increments with this compound were greater than with sodium salicylate. The rate of

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TABLE I. Effects of Iodinated Salicylates on Rats. Maximal change obtained after injection. Results given in terms of % change in basal metabolic rates with reference to determinations made on the same rat before injection.

Compound	Dosage, mg/100 g	% change in B.M.R. Normal	Hypo-metabolic	Hyper-metabolic
Sodium salicylate	12.8	+ 6.5	—	+ 23.4
	15.2	+ 40.3	+ 1.2	+ 25.2
	20.8	+ 28.7	+ 15.0	+ 70.5
	25.0	+ 39.4	- 23.8	+ 81.4
	30.0	+ 53.5	- 32.7	+ 75.6
5-Iodo-2-hydroxybenzoate	8.4	+ 14.2	—	+ 54.4
	11.5	+ 63.8	- 3.8	+ 80.5
	14.6	+100.5	+ 33.4	+ 95.4
	17.5	+159.2	+ 39.6	+155.7
	20.6	—	+ 80.0	—
5-Bromo-2-hydroxybenzoate	8.5	+ 10.6	—	+ 26.0
	11.5	+ 55.0	+ .4	+ 55.2
	14.5	+ 69.5	—	+ 90.3
	16.0	—	35.0	—
	17.5	116.9	—	+170.9
	20.4	—	+138.9	—
4-Iodo-2-hydroxybenzoate	8.5	+ 63.6	+ 5.7	—
	11.5	+ 60.4	+ 10.8	—
	14.5	+ 75.2	+ 53.8	—
	17.5	+136.7	—	—

TABLE II. Effects of Salicylates on Metabolism of Normal Mice. Maximal change after injection. Results given in terms of % change in metabolic rates with reference to determinations made on the same mouse before injection.

Compound	Dosage, mg/100 g	Change
Sodium salicylate	19.6	+ 22.2
	45.6	+ 28.2
	65.2	+ 37.2
	65.4	+ 28.6
	66.8	+ 40.1
	67.4	+ 37.8
	69.1	+ 33.8
5-Iodo-2-hydroxybenzoate	22.4	+ 33.9
	23.6	+ 26.9
	23.7	+ 32.6
	23.8	+ 80.0
	24.0	+ 14.6
	27.5	+ 30.0
	29.3	+100.0

change of metabolism with 5-iodo-2-hydroxybenzoate is very rapid with maximum change within 30 minutes, in contrast to maximum change with sodium salicylate, which may be 90 minutes. Comparable results were obtained with 5-bromo-2-hydroxybenzoate. 4-Iodo-2-hydroxybenzoate was also metabolically active in the normal and the thyroidectomized rat. Both sodium salicylate and 5-iodo-2-hydroxybenzoate produced increments in oxygen consumption of mice (Table II).

In Table III are given the results on the compounds which showed little or no activity. 3,5-Diiodo-2-hydroxybenzoate, 5-iodo-3-hydroxybenzoate, and 3-hydroxybenzoate did not increase the metabolic activity of rats and at the higher dose levels decreased metabolic activity.

Although no separate toxicity studies were made of the halogenated salicylates, our impression was that they were more toxic than sodium salicylate. There were several deaths in this series when the higher dosages of the halogenated compounds were used.

Summary. A study was made of the effects on oxygen consumption of halogenated salicylates in normal, thyroidectomized and hypermetabolic rats. It was found that 5-iodo-2-hydroxybenzoate and 5-bromo-2-hydroxybenzoate increased the metabolism of rats under these 3 conditions. 4-Iodo-2-hydroxybenzoate increased the metabolism of normal and thyroidectomized rats. 5-Iodo-2-hydroxy-

TABLE III. Effects of Iodinated Salicylates on Rats. Maximal change obtained after injection. Results given in terms of % change in basal metabolic rates with reference to determinations made on the same rat before injection.

Compound	Dosage, mg/100 g	% change in B.M.R. Normal	Hypo-metabolic	Hyper-metabolic
3-Hydroxybenzoate	40.3	- 11.1	—	—
	50.2	- 26.3	—	—
	70.3	- 41.2	- 40.1	- 39.2
	90.3	- 31.5	—	—
	110.3	- 39.6	- 38.1	- 41.3
	130.1	- 30.5	—	—
	150.1	- 33.3	—	- 23.8
5-Iodo-3-hydroxybenzoate	8.5	+30.3	—	+21.6
	11.5	- 30.1	—	- 18.1
	14.5	- 16.0	—	- 14.1
	17.5	- 21.7	—	- 2.6
	20.6	+ 7.5	—	+ 8.5
3,5-Diiodo-2-hydroxybenzoate	1.0	- 29.1	+ 8.5	- 18.3
	2.6	- 18.5	—	—
	3.0	—	- 25.9	- 9.5
	5.6	- 40.2	- 12.8	+17.6
	8.5	- 32.9	—	+32.6
	11.5	—	- 45	- 27.7
	14.5	- 30.9	- 31.2	—
	17.6	- 29.1	- 4.1	—
	20.6	—	- 40.9	—

benzoate and sodium salicylate increased the metabolism of normal mice. 3,5-Diiodo-2-hydroxybenzoate, 5-iodo-3-hydroxybenzoate and 3-hydroxybenzoate did not increase the metabolism of rats.

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Fecal Enzymes of Dogs with Pancreatic Exclusion.* (27415)

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It has recently been shown that the feces of rats contain enzymes that act on substrates digested by pancreatic enzymes(1). The present study was made to determine what portions of fecal enzymatic activity are of pancreatic origin by measuring the enzymatic activity in feces of normal dogs and of dogs in which pancreatic juice no longer entered the intestine.

Methods. Dogs. The studies were made on normal dogs and dogs in which a total separation of the pancreas from the duodenum, with interposition of omentum between the 2 organs, had been performed 6 to 8 weeks earlier. Both groups of animals were fed a commercial canned dog food *ad libitum*.

Feces. Feces were collected within 1 hour after passage. A portion of the sample was weighed, 0.9% sodium chloride solution was added to give a concentration of 0.1 g wet weight of feces per ml of final mixture, and homogenization was performed for 1 minute in a high speed blade-type homogenizer (Servall Omnimixer). The homogenate was stored in an ice bath until enzyme determinations were performed (within 3 hours of time of collection of the feces).

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Enzyme determinations. Enzyme determinations were made titrimetrically using a recording pH-stat (Radiometer). p-Toluene sulfonyl-L-arginine methyl ester was the substrate for trypsin, acetyl L-tyrosine ethyl ester for chymotrypsin, and vegetable oil emulsion for lipase. A complete description of the methods has been published(1). Enzymatic activity was expressed as micromoles of substrate split per minute per gram wet weight of feces. When required to bring the activity within the range of the methods fecal homogenates were diluted with 0.9% sodium chloride solution.

Results. Single samples of feces from 22 normal dogs were tested. All samples had readily measurable activity on all 3 substrates, but there was a wide range (Table I). Three dogs with pancreatic separation were studied and 10 samples of feces from each of these dogs were analyzed. Compared with the feces from normal dogs, the feces of the dogs with pancreatic separation had much lower enzymatic activities (Table I). Expressed as % of mean values for normal dogs the mean values for dogs with pancreatic separation were: lipase 17%, chymotrypsin 2%, and trypsin 4%. For lipase there was a slight overlap between the range of values for nor-