J. Pharm. Pharmac., 1968, **20**, 58–63 Received June 23, 1967

The distribution of salicylate in mouse tissues after intraperitoneal injection

J. A. STURMAN, P. D. DAWKINS, N. McARTHUR AND M. J. H. SMITH

The concentrations of salicylate and its principal metabolites were measured in blood, liver, brain, kidney, heart, spleen, diaphragm and skeletal muscle after the intraperitoneal injection of a fixed amount of radioactive salicylate and varying doses of unlabelled salicylate. The patterns of distribution of salicylate in the various organs with time were similar, the peak level being attained in 30–60 min after injection. Salicylate was eliminated from the blood after 8 hr but persisted in liver in measurable amounts up to 24 hr.

SALICYLATE inhibits a number of enzyme systems *in vitro*, including aminotransferases, dehydrogenases and oxidative phosphorylation reactions (Smith & Smith, 1966). The degree of inhibition of any particular enzyme depends on several factors, one of the most important being the concentration of salicylate in the reaction mixture. Similar considerations apply to the possible *in vivo* actions of the drug on tissue enzymes. A knowledge of the tissue concentrations of salicylate occurring after the administration of the drug is therefore a necessary preliminary to any studies designed to investigate the *in vivo* interactions of salicylate and enzyme systems. The present work describes the measurement of the concentrations of salicylate in mouse tissues at several time intervals after the injection of varying amounts of the drug.

Experimental

ANIMAL PREPARATION

Male mice, 25–30 g, of the albino strain maintained at King's College Hospital on M.R.C. modified cube diet No. 41B were divided into five groups, each of 45 animals. Each mouse received an intraperitoneal injection (0·2 ml) containing 2 μC of [^{14}C]salicylic acid (specific activity 15·5 mc per mmole, obtained from the Radiochemical Centre, Amersham, Bucks) plus sodium salicylate at a dose level of either 50, 100, 200, 400 or 800 mg/kg body weight. The injections were adjusted by adding sufficient sodium chloride to contain the same final concentration of sodium. Subgroups, each of five mice, were killed by cervical fracture at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 18 hr and 24 hr after injection. Food was withdrawn during the experiment but the animals were allowed to drink freely. Blood samples were collected, after decapitation, into lithium heparin tubes and the liver, brain, kidneys, spleen, heart, diaphragm muscle and both quadriceps femoralis muscles were rapidly removed, weighed on a torsion balance and stored in 80% (v/v) ethanol at −20°.

DETERMINATION OF TOTAL RADIOACTIVE MATERIAL IN THE TISSUE

The excised tissues were homogenized in 5 ml of 80% (v/v) ethanol using a Potter all-glass homogenizer. The homogenate was transferred to a centrifuge tube and the homogenizer washed out with a further 5 ml

From the Biochemical Pharmacology Department, King's College Hospital Medical School, Denmark Hill, London, S.E.5, England.

of the ethanol. The combined homogenate and washings were heated to 80° with frequent stirring and centrifuged at 2000 *g* for 30 min. The supernatant was decanted and the residue was resuspended in 5 ml of 80% (v/v) ethanol and the extraction repeated a further eight times with liver and five times with the other tissues. The solvent from the combined supernatants of each tissue was removed in a rotary evaporator at 55° under reduced pressure and the final residue dissolved in 5 ml of 80% (v/v) ethanol. Aliquots (0·5 ml) were used for the determination of total radioactivity in a Beckman liquid scintillation counter using as phosphor 5 ml of 0·4% 2,5-diphenyloxazole, 0·02% 1,4-bis-2-(4-methyl-5-phenyl-oxazolyl)-benzene and 60% naphthalene in 1,4-dioxan. The counting efficiency, determined by using [^{14}C]toluene as an internal standard, was 92 $\pm$ 2% for all the samples. Each sample was counted in duplicate until at least 10,000 counts were recorded.

DETECTION AND MEASUREMENT OF METABOLITES OF SALICYLATE

The radioactive substances present in the ethanolic solution of the final residue (see above) were separated by ascending chromatography, using Whatman No. 4 chromatography paper and benzene–acetic acid–water (4:2:2) and located by radioautography, using Kodak Blue Brand X-ray film and an exposure of 3 weeks. Radioactive salicylic acid occurred at the solvent front and its identity, and that of salicyluric acid (Rf = 0·35), were confirmed by co-chromatography with authentic material in a variety of solvent systems. Most of the chromatograms also showed a radioactive spot at the origin and this metabolite behaved as a single substance in a number of solvents. It was presumptively identified by its chromatographic position as a mixture of salicylglucuronides and this was confirmed by acid hydrolysis which gave a mixture of salicylic and glucuronic acids (see Quilley & Smith, 1952). The radioactivity in the separated metabolites was measured directly on the chromatography paper by a Packard Tri-Carb liquid scintillation counter using as phosphor 10 ml of 0·4% 2,5-diphenyloxazole and 0·01% 1,4-bis-2-(4-methyl-5-phenyl-oxazolyl)-benzene in toluene. The salicylate was completely extracted by the phosphor but salicylurate and the salicylglucuronides were insoluble and remained on the paper. Appropriate corrections were therefore made for the differing counting efficiencies.

PROTEIN BINDING OF SALICYLATE BY MOUSE PLASMA

The binding curve of salicylate and the proteins of human and mouse plasma were determined by the equilibrium dialysis method of Davison & Smith (1961) using [^{14}C]salicylic acid and measuring the radioactivity outside the dialysis sac before and after equilibration for 3 hr at 37° by the Beckman scintillation counter.

Results

Salicylate concentrations in mouse tissues between 5 min and 24 hr after the injection of doses ranging from 50 to 800 mg/kg are given in Table 1. The values for the highest dose are incomplete since the mice

TABLE 1. DISTRIBUTION OF SALICYLATE IN MOUSE TISSUES. The values are the means of five animals and are expressed as mg/litre for blood and mg/kg wet weight for the other tissues.

Tissue	Dose injected (mg/kg)	Time after injection								
		5 min	15 min	30 min	1 hr	2 hr	4 hr	8 hr	18 hr	24 hr
Blood	50	32·8	41·7	51·1	26·9	8·1	1·3	0	0	0
	100	53·2	84·6	102·1	63·3	30·1	6·1	0	0	0
	200	122·6	167·9	174·6	121·9	98·9	11·7	1·9	0	0
	400	291·6	291·4	387·7	233·1	162·7	37·4	22·9	0	0
	800	675·9	564·4	604·7	491·7	205·0	—	—	—	—
Liver	50	8·5	10·6	22·5	21·5	13·9	4·4	0·5	0	0
	100	24·0	44·0	62·9	46·5	41·6	14·6	1·3	0·1	0
	200	92·0	120·5	143·6	111·4	85·0	42·4	4·8	2·9	1·3
	400	173·3	198·6	270·4	227·6	178·4	96·8	23·3	13·0	6·4
	800	282·8	303·1	325·8	258·5	208·4	—	—	—	—
Brain	50	4·5	9·4	9·1	5·3	1·6	0·3	0	0	0
	100	6·8	23·4	24·4	15·9	7·3	0·8	0	0	0
	200	17·5	48·0	53·0	40·0	32·9	4·1	1·4	0·2	0
	400	47·3	67·5	123·9	95·9	91·3	32·0	6·3	1·0	0
	800	104·0	177·0	226·0	238·7	224·0	—	—	—	—
Kidney	50	23·8	28·4	37·7	33·0	12·8	2·2	0·4	0	0
	100	40·0	51·9	62·9	48·2	32·5	5·9	0·8	0	0
	200	75·1	90·5	100·9	92·8	74·4	21·0	11·3	0·2	0
	400	130·4	160·6	255·9	174·0	160·4	66·0	36·4	0·9	0·5
	800	263·6	281·0	412·5	331·1	269·1	—	—	—	—
Heart	50	12·8	15·9	16·2	9·0	3·7	0·6	0·4	0	0
	100	27·0	39·5	37·9	30·4	17·1	1·8	0·9	0	0
	200	66·8	79·9	78·2	67·5	57·1	8·0	3·0	0	0
	400	122·3	149·0	192·5	187·8	139·8	48·0	18·7	0	0
	800	176·3	235·2	274·4	245·4	162·8	—	—	—	—
Spleen	50	8·1	10·7	12·6	8·5	4·0	0·6	0	0	0
	100	19·7	27·9	31·9	26·4	17·7	2·0	0	0	0
	200	49·3	63·5	71·8	61·6	47·4	5·9	0·1	0	0
	400	105·6	133·8	173·8	150·5	123·9	21·5	1·3	0	0
	800	189·1	232·2	256·7	278·1	141·4	—	—	—	—
Diaphragm	50	10·8	12·0	21·6	10·2	8·0	1·9	1·4	0	0
	100	28·7	33·7	45·7	34·3	30·6	4·6	2·9	0	0
	200	75·0	75·2	79·5	91·7	75·4	12·1	8·9	1·5	0
	400	473·1	222·0	204·5	221·9	196·9	33·4	32·7	5·5	0
	800	904·4	336·2	248·2	227·4	175·1	—	—	—	—
Muscle	50	5·6	7·4	9·5	5·8	2·7	1·0	0·3	0	0
	100	7·2	12·4	17·7	16·2	14·5	2·1	0·8	0	0
	200	22·2	37·8	44·8	39·2	38·2	26·6	2·5	0	0
	400	74·7	93·1	123·4	106·3	88·0	74·6	8·5	0	0
	800	205·7	238·5	271·3	317·0	157·5	—	—	—	—

died 2–4 hr later. No deaths occurred in the other groups. The blood salicylate reached a peak at 30 min, except for the 800 mg/kg dose when it was reached at 5 min. Salicylate disappeared from the circulation between 4 and 8 hr with the two lowest doses and between 8 and 18 hr with the 200 and 400 mg/kg doses. The distribution of the salicylate with time in the other tissues followed a similar pattern except that very high concentrations of salicylate were observed in the diaphragm muscle at 5 min after the injection of the high doses (400 and 800 mg/kg) and that the salicylate persisted in some of the tissues, particularly the liver, up to 24 hr. One possible explanation for these initial high levels of salicylate in the diaphragm is that the drug entered the tissue directly from the peritoneal cavity and not via the circulation during a time interval when the salicylate concentration in the peritoneal fluid was at its maximum.

Gentisic acid and other hydroxylated metabolites of salicylate were not detected in any tissue. Salicylurate was found only in kidney and appeared

in samples obtained 30 min after injection to the extent of 3 to 7% of the dose of salicylate but was not detected in animals killed 2 hr or more after injection. Salicylglucuronides were present in all the tissues except brain. The concentrations of glucuronide present varied with time similarly in all tissues. The glucuronides accumulated for the first 15 min, then the concentrations fell sharply and slowly recovered over the next 2 hr, finally decreasing to zero between 2 and 24 hr. This effect was most pronounced in the liver and is illustrated in Fig. 1.

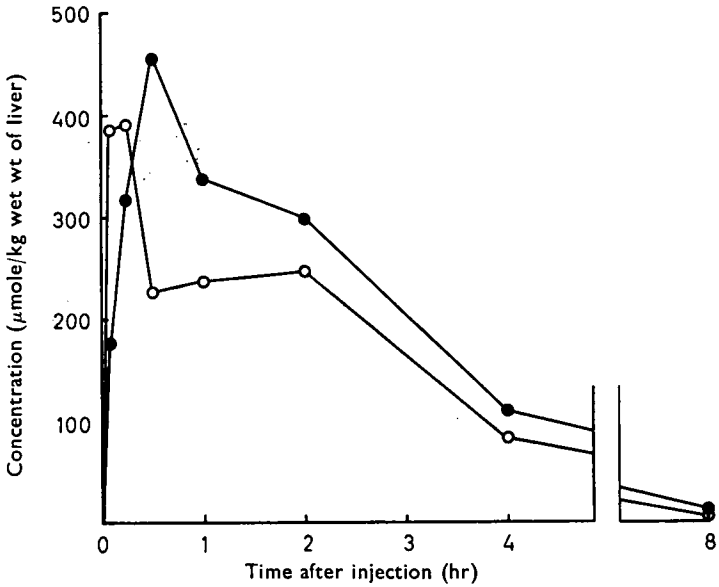

FIG. 1. Concentration of salicylate and salicylglucuronide in mouse liver after the intraperitoneal injection of 100 mg/kg body weight of sodium salicylate. ●, Salicylate; ○, salicylglucuronides.

Discussion

Many workers have studied the plasma salicylate concentrations in man and animals after single or repeated doses of the drug. However, apart from blood and urine, little information is available about the distribution of salicylate and its metabolites in tissues. Fragmentary data have been published about the levels of salicylate in human organs in cases of suicide, accidental poisoning and death during salicylate therapy and isolated reports have appeared giving values for a few animal tissues (Gross & Greenberg, 1948). The most comprehensive experiments have been done with the rat. Smith, Gleason & others (1946) determined the salicylate levels in liver, muscle, kidney, brain and lung after the oral administration of the drug. They found that the salicylates in the tissue fluid of the liver, kidneys and lung was approximately the same as that in the serum while the corresponding levels in muscle and brain were much lower. The distribution of radioactive salicylate and its metabolites in many rat organs

at 0·5, 1, 2 and 4 hr after the intraperitoneal injection of [^{14}C]salicylate was investigated by Wolff & Austen (1958). The principal findings were that the peak level of salicylate occurred 1 hr after the injection and 90–95% of the radioactivity in the tissues was salicylate itself. Traces of salicylglucuronides and salicylurate were detected in all tissues, except brain and thyroid gland, and the liver and kidney contained 2–6% of the radioactivity as glucuronides and 1–2% as gentisic acid.

The present results (Table 1) give a more complete picture of the distribution of salicylate in mouse tissues at several time intervals after the intraperitoneal injection of varying doses of salicylate. The patterns of distribution of salicylate in the various organs with time were similar in all the tissues examined, the peak level being attained in 30–60 min after the injection. Salicylate was eliminated from the blood after 8 hr but persisted in the liver, in measurable amounts, up to 24 hr.

The plasma salicylate concentrations found in the mouse were much lower than would have been predicted on the basis of similar experiments in man. Thus a single dose of 2 g of sodium salicylate (equivalent to 30 mg/kg body weight) in man gives plasma salicylate concentrations varying between 75 and 200 mg/litre (Smith & others, 1946). The 50 mg/kg dose in the mouse only produced a concentration of 50 mg/litre at the peak level and the values at the other time intervals were much lower (Table 1). The main factor which determines circulating salicylate levels is the extent to which salicylate is bound to the serum proteins, particularly albumin (Reynolds & Cluff, 1960). In man, the relative extent of the binding decreases with increasing salicylate concentration (Davison & Smith, 1961) and the present work shows that this is also true for the mouse except that the amount of salicylate bound at a given salicylate concentration is much less in the latter species (Fig. 2).

This finding could explain the relatively low circulating total salicylate concentrations observed in the mouse plasma samples. It also implies that the elimination of salicylate from the blood, i.e. the half-life of circulating salicylate, should be lower in the mouse than in man. Done (1960) has shown that the regression curve for plasma salicylate levels in man quickly assumes the characteristics of a first-order reaction and that the levels declined at a reasonably constant fractional rate. It is possible to derive a value for the mean slope of this regression curve and hence to calculate the average half-life for circulating salicylate in man to be 20 hr (Done, 1960). A similar calculation for the mouse, using the present results, gives a value of 1 hr for the half-life of circulating salicylate.

Salicylglucuronides were detected in all the mouse organs except the brain. It has been shown by Schachter, Kass & Lannon (1959) that the liver, kidney, lung, spleen and urinary bladder of many animal species are able to conjugate salicylate to salicylglucuronides *in vitro* but that other tissues, including the brain, were unable to effect the biosynthesis of the salicylglucuronides. These results suggest that the occurrence of salicylglucuronides in mouse skeletal muscle, diaphragm and heart is due to entry of the conjugates from the circulation after their biosynthesis in the other mouse tissues. The present work also shows that the circulating

glucuronides do not enter the mouse brain and that the blood-brain barrier must be impermeable to salicylglucuronides.

The concentrations of circulating salicylglucucuronides fluctuated with time in a different manner to the concentrations of circulating salicylate (Fig. 1). One possible explanation is that during the initial phase of the experiments (0–15 min after injection) the biosynthesis of the

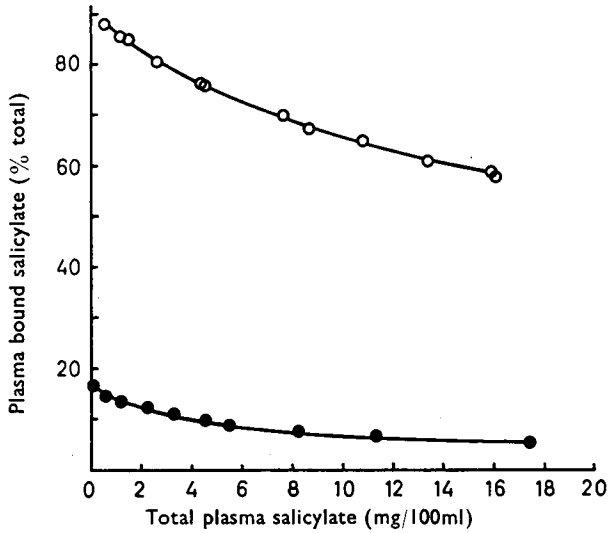

FIG. 2. Protein binding curves of salicylate in human and mouse plasma. ○, human plasma; ●, mouse plasma.

glucuronides in the liver and other tissues proceeded normally. However, when the liver salicylate concentration increased to the peak level (15–60 min) the biosynthesis of the glucuronides was partially inhibited due to the uncoupling action of salicylate on oxidative phosphorylation processes interfering with energy-dependent synthetic reactions. The concentrations of salicylglucuronide in the liver were therefore decreased during this period and their levels only started to rise again when the salicylate concentrations decreased after the peak level.

Acknowledgements. We wish to thank the Arthritis and Rheumatism Council and King's College Hospital and Medical School Research Committee for financial support, and Miss M. Skinner for technical assistance.

References

Davison, C. & Smith, P. K. (1961). *J. Pharmac. exp. Ther.*, **133**, 161–170.
Done, A. K. (1960). *Pediatrics*, **26**, 800–807.
Gross, M. & Greenberg, L. A. (1948). *The Salicylates*, pp. 48–50, New Haven: Hillhouse Press.
Quilley, E. & Smith, M. J. H. (1952). *J. Pharm. Pharmac.*, **4**, 624–630.
Reynolds, R. C. & Cluff, L. E. (1960). *Bull. Johns Hopkins Hosp.*, **107**, 278–290.
Schachter, D., Kass, D. J. & Lannon, T. J. (1959). *J. biol. Chem.*, **234**, 201–205.
Smith, P. K., Gleason, H. L., Stoll, C. G. & Orgorzalek, S. (1946). *J. Pharmac. exp. Ther.*, **87**, 237–255.
Smith, M. J. H. & Smith, P. K. (1966). *The Salicylates*, pp. 49–105, New York: Interscience Publishers.
Wolff, J. & Austen, F. K. (1958). *J. clin. Invest.*, **37**, 1144–1152.