Br. J. Pharmac. (1984), **82**, 241–248

Influence of age, sex, pregnancy and protein-calorie malnutrition on the pharmacokinetics of salicylate in rats

D.R. Varma & T.L. Yue

Department of Pharmacology & Therapeutics, McGill University, 3655 Drummond Street, Montreal, Canada H3G 1Y6

1 The influence of age, sex, pregnancy and protein-calorie malnutrition (PCM) on the plasma $t_{\frac{1}{2}}$, plasma clearance (Cl_p) and apparent volume of distribution (V_d) of sodium salicylate (62 μmol kg^{-1}) was determined in Sprague-Dawley rats. Female and male rats of five different age groups (ages in weeks: pups 1, weanling 3, young 8–9, adult including pregnant 14–15, old 56–60) including three age groups with PCM (8–9, 14–15 and 56–60 weeks old) were used. Plasma and urinary salicylates were assayed by h.p.l.c.

2 Plasma $t_{\frac{1}{2}}$ was longer and Cl_p smaller in pups than in weanling and young rats and comparable to values for old rats; V_d of salicylate in pups was larger than in any other group of rats. Plasma $t_{\frac{1}{2}}$ was longer and Cl_p as well as V_d of salicylate were smaller in adult females than in males of comparable age. Relative to nonpregnant adult females, V_d of salicylate in pregnant rats was larger but plasma $t_{\frac{1}{2}}$ and Cl_p were unchanged.

3 In all groups of rats studied, PCM decreased the plasma $t_{\frac{1}{2}}$ and increased the Cl_p of salicylate; V_d was unchanged.

4 Changes in salicylate pharmacokinetics were not due to any differences in serum protein-salicylate binding or to serum testosterone levels. Ovariectomy decreased the plasma $t_{\frac{1}{2}}$ of salicylate but castration of male rats had no significant effect. Administration of testosterone to ovariectomized female rats exerted no significant effect on salicylate pharmacokinetics.

5 It is concluded that the physiological state and the nutritional status can modify salicylate pharmacokinetics; in so far as the rat model reflects the human situation, these variables should be taken into account for a rational salicylate therapy.

Introduction

Age (O'Malley *et al.*, 1971; Schmucker, 1979), sex (O'Malley *et al.*, 1971; Giudicelli & Tillement, 1977), pregnancy (Krauer & Krauer, 1977) and protein-calorie malnutrition (Krishnaswamy, 1978; Varma, 1981) are well recognized determinants of drug metabolism and pharmacokinetics. An influence of age and nutritional status on the disposition of salicylate has also been reported. Salicylate elimination was found to be slower in human (Levy & Garrettson, 1974) and animal (Davis *et al.*, 1973) neonates and in the elderly (Cuny *et al.*, 1979; Montgomery & Sitar, 1981) than in adults. The decline in plasma salicylate concentration with time appeared to be slower in pregnant rats (Varma & Yue, 1983) than in male rats (Yue & Varma, 1982) suggesting that pregnancy or sex might influence salicylate pharmacokinetics. We have previously shown that protein-calorie malnutrition in rats significantly decreased the plasma half-life of salicylate (Yue & Varma, 1982). Because salicylates are one of the most commonly used drugs (Flower *et al.*, 1980) and protein-calorie malnutrition is widespread in all age groups, especially in developing countries (see, Varma, 1981), we studied the influence of various physiological states and of protein deficiency on the pharmacokinetics of salicylate in rats as the experimental model.

Methods

Animals and diet

Sprague-Dawley female and male rats weighing between 100–125 g (5–6 weeks old) or 200–225 g

(10–11 weeks old) were purchased from Canadian Breeding Farms, St. Constant, Quebec, Canada and maintained in the McIntyre Animal Centre of McGill University. Pregnant rats and 1-, 3-, and 50–60-week old rats were raised from these animals in the Animal Centre. Three weeks before studies on salicylate pharmacokinetics, all animals except the 1- and 3-week old rats were placed individually in wire-bottomed suspended cages and provided with water and a 21% (control) or a 5% (low) protein diet *ad libitum*; the latter groups of animals are designated as suffering from protein-calorie malnutrition (PCM) (Varma, 1979).

The composition of the control 21% protein diet was as follows ($g\,kg^{-1}$): vitamin-free casein 231, sucrose 519, corn starch 150, corn oil 50, mineral mixture (Williams-Briggs) 40 and vitamin mixture (Teklad) 10. The 5% protein diet contained 55 $g\,kg^{-1}$ vitamin-free casein and 695 $g\,kg^{-1}$ sucrose; all other constituents were identical to those in the control diet. Both diets were isocaloric in composition and purchased from Teklad Test Diets, Madison, Wisconsin, U.S.A.

Further details on the housing of animals and raising the pregnant rats are provided elsewhere (Varma, 1979; Varma & Yue, 1983).

Injections and collection of samples

Intravenous injections (into all animals except the 1- and 3-week old rats), collection of blood samples from tail arteries (Varma 1979) and hearts (all animals except 1-week old pups) and castrations were all performed under ether anaesthesia. Intraperitoneal injections were made into unanaesthetized 1- and 3-week old animals. Blood from 1-week old pups was collected following decapitation. Animals were placed individually in metabolic cages for the collection of urine samples.

Pharmacokinetic studies

Sodium salicylate (62 $\mu mol\,kg^{-1}$) was injected and blood samples (0.1–0.2 ml) were collected from the tail artery (8–60 weeks old rats) at 1, 3, 6, 9, 12 and 24 h or from the heart (3-week old rats) at 2, 6, 12 and 24 h following the injection. In the case of 1-week old pups, blood samples were not collected serially; instead one pup was decapitated at 1, 3, 6, 9, 12 and 24 h following the injection and blood was collected. Five to six female and male pups from a single litter constituted one experiment. For the collection of urine samples, 62 $\mu mol\,kg^{-1}$ sodium salicylate was injected intravenously and animals were placed in metabolic cages; 0–3, 3–6, 6–12 and 12–24 h urine samples were collected for the extraction and assay of salicylates (Yue & Varma, 1982).

Plasma salicylate was assayed in salicylic acid equivalents and data were fitted according to the method of least squares in order to derive plasma half-life ($t_{\frac{1}{2}}$), apparent volume of distribution (V_d) and plasma clearance (Cl_p) as described previously (Varma, 1979; Yue & Varma, 1982). Urinary salicylate data were computed as cumulative excretion of total salicylate as well as individual metabolites (Yue & Varma, 1982).

Castration and testosterone administration

Ovariectomy or orchidectomy was performed 4–5 weeks before the study of salicylate pharmacokinetics. Into a group of ovariectomized animals, testosterone-containing 25 mm long polydimethylsiloxane tubes (kindly provided by Dr B. Robaire of authors' Department) were implanted under the skin at the time of the surgery in order to deliver approximately 260 nmol of the hormone per day (Robaire *et al.*, 1979).

Serum protein-salicylate binding

Serum samples from adult and old female and male rats were incubated with sodium salicylate (360 μM) at 37 °C for 1 h following which the fractional binding was determined by ultrafiltration using Amicon Centriflow membrane cones (CF 50A) (Yue & Varma, 1982).

Extraction and assay of salicylates

Salicylic acid and metabolites in serum and urine were extracted in benzene: ethyl acetate (1:1, v/v) after acidification of the samples with one drop of 85% H_3PO_4 and assayed by high-pressure liquid chromatography (Peng *et al.*, 1978) according to the following conditions: a reverse-phase column (μBondapak-C_{18}, Waters Associates, Milford, Massachussets, U.S.A.), a mobile phase of 30% acetonitrile in 0.05% H_3PO_4 at a flow rate of 1 $ml\,min^{-1}$ and an Altex h.p.l.c. pump. Phthalic acid was used as the internal standard for serum samples and urine samples were quantitated on the basis of standard curves to authentic compounds (Yue & Varma, 1982). Aliquots of each urine sample were incubated with β-glucuronidase (500 units ml^{-1} urine) at 37 °C and pH 5 for 15 h; the increase in urinary salicylic acid after the incubation was taken to represent salicyl glucuronides (Yue & Varma, 1982).

Other determinations and statistics

Serum testosterone (possibly androgens) was measured by means of radioimmunoassay kits (Bio-RIA, Louisville, Kentucky, U.S.A.) Proteins were assayed

Table 1 Body weights, serum proteins and serum testosterone levels in rats of different ages

Age (weeks)	Group[a]	Female body weights (g)	Male body weights (g)	Female Serum proteins ($g\,dl^{-1}$)	Male Serum proteins ($g\,dl^{-1}$)	Male Serum testosterone ($pmol\,ml^{-1}$)
1	pups	12±0.2	13±0.1	4.4±0.2	4.3±0.2	2.2±0.4
3	weanling	56±1.5	56±1	5.4±0.2	5.2±0.1	0.6±0.2
8–9	young	189±3	253±3[b]	7.4±0.3[c]	7.5±0.1[c]	12.2±2.0[c]
14–15	adult	260±10	334±4[b]	8.0±0.3[c]	7.4±0.1[c]	13.1±2.3[c]
14–15	pregnant	338±7	—	7.5±0.3	—	—
50–60	old	360±9	595±35[b]	9.0±0.3[c]	8.5±0.3[c]	7.0±1.2[c]

Values are means ± s.e.; $n = 5–15$
[a]These group designations in the text refer to ages shown against them.
[b]Different ($P < 0.05$) from the corresponding values for female animals.
[c]Different ($P < 0.05$) from the top 2 values in the same column.

according to Lowry *et al.* (1951) with bovine serum albumin as the standard. Differences between means were ascertained by Student's *t* test and a probability of less than 0.05 was assumed to denote a significant difference. Data are presented as the mean ± s.e.

Chemicals

The following chemicals were purchased: phthalic acid, salicylic acid and sodium salicylate (BDH, Montreal, Canada); bovine serum albumin, β-glucuronidase (Glucurase, bovine liver) and salicyluric acid (Sigma Chemical Co., St. Louis, Missouri, U.S.A.); acetonitrile, benzene, ethyl acetate of high purity grade (Fisher Scientific, Montreal, Canada).

Results

Animal characteristics

With the exception of 1- and 3-week old rats, body weights of female rats were lower than those of male rats of comparable age (Table 1); protein-calorie malnutrition (PCM) led to a decrease in body weight gain (Tables 3 and 4). Serum proteins increased with age (Table 1) but in no age group there were significant differences in serum protein levels of female and male rats. Testosterone levels rose markedly after 3 weeks of age in male rats (Table 1); no significant difference in serum testosterone levels of young, adult and old male rats was found. Effects of castration on serum testosterone levels are shown in Table 5; serum testosterone levels were below the level of the sensitivity of our assay procedure in uncastrated adult female rats as well as in castrated male rats. The implantation of testosterone-containing Silastic tubes into ovariectomized female rats resulted in serum testosterone levels of 5.6 ± 1.2 $pmol\,ml^{-1}$ (Table 5).

Influence of age and sex on salicylate pharmacokinetics (Table 2)

Following injections of sodium salicylate, salicylic acid was the only compound detectable in serum of all groups of animals studied. Compared to all other

Table 2 Pharmacokinetics of sodium salicylate ($62\,\mu mol\,kg^{-1}$) in female and male rats of different ages

Age (weeks)	Female $t_{\frac{1}{2}}$ (h)	Male $t_{\frac{1}{2}}$ (h)	Female V_d ($ml\,kg^{-1}$)	Male V_d ($ml\,kg^{-1}$)	Female Cl_p ($ml\,kg^{-1}\,h^{-1}$)	Male Cl_p ($ml\,kg^{-1}\,h^{-1}$)	Female C_p ($nmol\,ml^{-1}$) at 6 h	Male C_p ($nmol\,ml^{-1}$) at 6 h
1	12.0±1.8	13.9±1.9	391±31[a]	433±70[a]	25.0±5.0	22.0±4.0	100–150	125–150
3	2.7±0.2[a]	2.5±0.2[a]	201±21	149±16	54.0±4.0[a]	44.0±3.0[a]	50–81	50–113
8–9	7.3±0.3[a]	6.6±0.7	144± 8	213±18[b]	13.8±1.2	23.2±2.1[b]	250–306	144–209
14–15	11.9±0.7	7.1±0.6[b]	150±10	186±15[b]	8.9±0.7	18.6±1.6[b]	156–388	125–250
56–60	15.7±1.4	10.4±2.3	175±16	165±19	7.9±0.8	13.5±2.1[b]	206–356	188–269

Values are mean ± s.e.
V_d = apparent volume of distribution; Cl_p = plasma clearance; C_p = plasma salicylic acid concentration.
[a]Different ($P < 0.05$) from all other values in the same column.
[b]Different ($P < 0.05$) from the corresponding value for the female rats.

Table 3 Influence of protein-calorie malnutrition (PCM) on the pharmacokinetics of sodium salicylate (62 μmol kg^{-1}, i.v.) in nonpregnant and pregnant (day 20 of gestation) female rats

Age (weeks)	*Group*	*Body weight* (g)	$t_{\frac{1}{2}}$ (h)	V_d (ml kg^{-1})	Cl_p (ml kg^{-1} h^{-1})
8–9	Control	189 ± 3	7.3 ± 0.3	144 ± 18	14 ± 1.2
8–9	PCM	118 ± 5[a]	3.3 ± 0.2[a]	138 ± 8	29 ± 1.6[a]
14–15	Control	260 ± 10	11.9 ± 0.7	150 ± 10	9 ± 0.7
14–15	PCM	202 ± 5[a]	7.1 ± 0.8[a]	152 ± 9	15 ± 0.8[a]
14–15	Control pregnant	338 ± 7	12.9 ± 1.4	212 ± 14[b]	12 ± 1.8
14–15	PCM pregnant	233 ± 4[a]	8.1 ± 1.5[a]	243 ± 22[b]	23 ± 3.5[a]
56–60	Control	360 ± 9	15.7 ± 1.4	175 ± 16	8 ± 0.8
56–60	PCM	331 ± 10[a]	6.6 ± 1.0[a]	147 ± 39	15 ± 1.4[a]

Values are mean ± s.e. of 5–11 experiments.
V_d = apparent volume of distribution; Cl_p = plasma clearance.
[a]Different ($P < 0.05$) from the control value (immediate top).
[b]Different ($P < 0.05$) from the value for nonpregnant adult female rats.

groups of rats, the plasma half-life ($t_{\frac{1}{2}}$) was shortest and clearance (Cl_p) greatest in the 3-week old animals. The volume of distribution (V_d) was larger in 1-week old pups than in any other group of rats. From the weanling to 50–60 weeks of age, there was a trend towards an increase in $t_{\frac{1}{2}}$ and a decrease in Cl_p in both female and male rats but these changes were significant only in female rats. All pharmacokinetic parameters ($t_{\frac{1}{2}}$, V_d, Cl_p) of salicylate in adult female rats were significantly different from those in young or adult male rats.

Salicylate pharmacokinetics in pregnant rats

Plasma $t_{\frac{1}{2}}$ and Cl_p of salicylate in pregnant rats were not different from the values in adult females of comparable age; the volume of distribution, however, was greater in pregnant than in adult female rats (Table 3).

Protein-calorie malnutrition and salicylate pharmacokinetics

PCM decreased the plasma $t_{\frac{1}{2}}$ and increased the Cl_p of salicylate in all groups of rats studied; data for nonpregnant and pregnant female animals are shown in Table 3 and for male rats in Table 4. PCM did not significantly alter the volume of distribution of salicylate.

Urinary excretion of salicylate

Because the pharmacokinetics of salicylate in adult female rats were different from those in young as well as adult male rats, urinary excretion of salicylate was determined only in adult female and adult male animals (Figure 1). Urinary excretion of salicylic acid and salicyluric acid in the female and male rats did not differ. The excretion of total salicylate and salicyl

Table 4 Influence of protein-calorie malnutrition (PCM) on the pharmacokinetics of sodium salicylate (62 μmol kg^{-1}, i.v.) in male rats

Age (weeks)	*Group*	*Body weight* (g)	$t_{\frac{1}{2}}$ (h)	V_d (ml kg^{-1})	Cl_p (ml kg^{-1} h^{-1})
8–9	Control	253 ± 3	6.6 ± 0.7	213 ± 18	23 ± 2.1
8–9	PCM	116 ± 2[a]	3.9 ± 0.3[a]	199 ± 17	38 ± 4.6[a]
14–15	Control	334 ± 4	7.1 ± 0.6	186 ± 15	19 ± 1.6
14–15	PCM	220 ± 6[a]	3.1 ± 0.7[a]	155 ± 13	38 ± 5.8[a]
56–60	Control	596 ± 35	10.4 ± 2.3	165 ± 19	14 ± 2.1
56–60	PCM	506 ± 149[a]	4.9 ± 0.8[a]	148 ± 8	22 ± 2.3[a]

Values are mean ± s.e. of 5–11 experiments.
V_d = apparent volume of distribution; Cl_p = plasma clearance.
[a]Different ($P < 0.05$) from the control value for the same age group.

Table 5 Influence of castration of female (F) and male (M) rats on the pharmacokinetics of sodium salicylate (62 μmol kg^{-1}, i.v.)

Age (weeks)	*Sex*	*Procedure*	*Body weight* (g)	*Serum testosterone* (pmol ml^{-1})	$t_{\frac{1}{2}}$ (h)	V_d (ml kg^{-1})	Cl_p (ml kg^{-1} h^{-1})
14–15	F	None	260 ± 10	0	11.9 ± 0.7	150 ± 10	9 ± 0.7
14–15	F	Ovariectomy	299 ± 17	not done	8.8 ± 0.9[b]	167 ± 14	14 ± 1.0[b]
14–15	F	Ovariectomy + testosterone[a]	318 ± 12	5.6 ± 1.2	9.4 ± 0.3[b]	154 ± 3	11 ± 0.5[b]
8–9	M	None	253 ± 3	12.2 ± 2.0	6.6 ± 0.7	213 ± 18	23 ± 2.1
8–9	M	Orchidectomy	233 ± 11	0	7.7 ± 0.8	171 ± 6[b]	17 ± 2.1

Values are means ± s.e. of 5–11 experiments.
V_d = apparent volume of distribution; Cl_p = plasma clearance.
[a] Administered by testosterone-containing Silastic tube implants.
[b] Different ($P < 0.05$) from the value for the unoperated animals of the same sex.

glucuronides in male rats was greater than in female rats although the difference was not statistically significant; the relatively greater excretion of total salicylate in male than in female rats could be almost entirely accounted for by the differences in salicyl glucuronides.

Castration and salicylate pharmacokinetics

Ovariectomy significantly decreased the plasma $t_{\frac{1}{2}}$ and increased the plasma clearance of salicylate such that the $t_{\frac{1}{2}}$ in these animals (adult females, age 14–15 weeks) was no more different from that in young (Table 5) or adult (Table 2) male rats. Administration of testosterone to ovarectomized rats did not exert any significant effect on the pharmacokinetics of salicylate. Also, castration of male rats did not change the $t_{\frac{1}{2}}$ and Cl_p of salicylate although it did lead to a decrease in the volume of distribution of the drug (Table 5).

Table 6 *In vitro* binding of salicylate to serum proteins from female (F) and male (M) rats

Age (weeks)	*Sex*	n	*Bound fraction*[a] mean ± s.e. (%)
14–15	F	6	87 ± 2.5
14–15	M	7	84 ± 3.5
56–60	F	4	76 ± 2.7[b]
56–60	M	7	84 ± 0.8

[a] Serum samples were incubated with 360 μM sodium salicylate at 37 °C for 1 h before determination of binding by ultrafiltration.
[b] Different ($P < 0.05$) from the values for 14–15 week old female and 56–60 week old male rats.

Serum protein-salicylate binding

The fractional binding of salicylate to serum proteins from adult female and male rats and old male rats did not differ. The binding to serum proteins from old female rats was less than to serum proteins from old male or adult female rats (Table 6).

Discussion

The main purpose of this study was to find out whether age, sex, pregnancy and protein-calorie malnutrition (PCM) influenced the pharmacokinetics of salicylate. For this purpose female and male rats of five different ages, pregnant rats and three groups of rats with PCM were used as the experimental model. The results of this study clearly show that the physiological state and the nutritional status significantly influence the pharmacokinetics of salicylate.

We have previously shown (Yue & Varma, 1982) in conformity with other reports (Nelson *et al.*, 1966) that the decline in plasma salicylate concentration in rats over a wide dose range (approximately 12–620 μmol kg^{-1}) proceeds according to a first-order kinetics. In the present study at a 62 μmol kg^{-1} dose level, plasma salicylic acid concentrations declined monoexponentially at least from 1 to 12 or 24 h in all groups of animals studied. Although in humans (Nelson *et al.*, 1966; Levy, 1979) as well as in rats (Nelson *et al.*, 1966; Yue & Varma, 1982) salicyluric acid formation is a saturable process as is also evidenced in the present study (Figure 1 depicting excretion of salicyluric acid at a constant rate in both female and male rats), the consequence of this on the overall elimination of salicylate in the two species is not identical. Salicyluric acid is a major

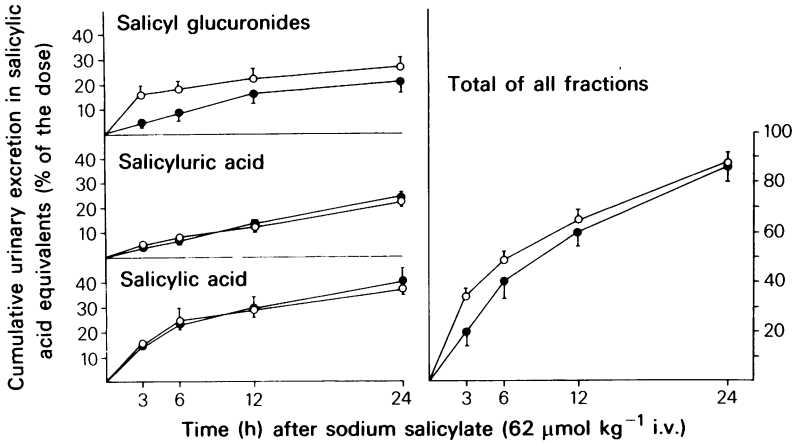

Figure 1 Cumulative urinary excretion of salicylic acid and metabolites in adult female (●) and male (○) rats. Each value is the mean of 5–7 animals; vertical lines show s.e.means.

metabolite of salicylate in man (Hutt *et al.*, 1982; Levy, 1979). In rats, on the other hand, salicyluric acid accounts for less than 30% of total urinary salicylates (Nelson *et al.*, 1966; Yue & Varma, 1982); in the present study also, salicyluric acid accounted for less than 25% of total salicylates in the urine (Figure 1). Our data are supportive of the suggestion that salicylate elimination by apparent first-order kinetics in rats is similar to the situation in man at high doses of salicylate when salicyluric acid becomes an insignificant fraction of total salicylates excreted by the kidney (Nelson *et al.*, 1966). Therefore an assessment of the influence of different physiological states on salicylate pharmacokinetics on the basis of plasma $t_{\frac{1}{2}}$ and clearance (Cl_p) and the volume of distribution (V_d) is reasonable. It should be pointed out, however, that even in animals with a relatively long plasma $t_{\frac{1}{2}}$ of salicylate (up to approximately 16 h), plasma samples were collected only up to 24 h after drug administration, which might have introduced errors in the derivation of plasma half-lives.

The finding that salicylate elimination was slower in 1-week pups than in weanling and young animals is in accordance with data for man (Levy & Garrettson, 1974; Garrettson *et al.*, 1975) and animal (Davis *et al.*, 1973) neonates. A relatively slow rate of salicylate elimination has been attributed to a poorly developed mechanism for the conjugation of salicylate with glycine and glucuronic acid (Davis *et al.*, 1973; Levy & Garrattson, 1974). It is reasonable to suggest that a similar mechanism is responsible for a relatively slow elimination of salicylate by rat neonates.

Surprisingly the elimination of salicylate in weanling rats was faster than in any other group of animals. The reason for this is unclear from our studies. However, other workers (Kato *et al.*, 1964) have reported faster metabolism of pentobarbitone, carisoprodol and meprobamate by 30-day old rats than by younger or older animals. Although the metabolism of these agents involves hydroxylation and that of salicylate conjugation (Levy, 1979; Hutt *et al.*, 1982), it is likely that salicylate conjugation is greater in weanling than in older rats and neonates.

Although sex is recognized to influence drug disposition (O'Malley *et al.*, 1971; Giudicelli & Tillement, 1977), such an influence on the disposition of salicylate, perhaps the most commonly used drug, has not been determined in any detail. Studies on the hydrolysis of aspirin by serum esterases of women have yielded conflicting data; Menguy *et al.* (1972) reported a decrease and Rainsford *et al.* (1980) found no difference in the aspirin esterase activity of serum from women. The present study demonstrates that any changes in the pharmacological effects of salicylate in females might be related to a relatively slow elimination of salicylic acid.

The observed increase in salicylate plasma $t_{\frac{1}{2}}$ in adult females cannot be attributed to any changes in its binding to serum proteins (Table 6). A smaller volume of distribution of salicylate in female than in male rats (Table 2) would be expected to decrease rather than increase the plasma $t_{\frac{1}{2}}$ of salicylate; however, the reverse was the case. Also the V_d in pregnant rats was larger than in adult females of comparable age but there was no difference in plasma $t_{\frac{1}{2}}$ of salicylate. These data suggest that changes in plasma $t_{\frac{1}{2}}$ of salicylate in female rats are due to factors other than any differences in V_d. We did find some decrease in the urinary excretion of salicyl glucuronides, which accounted for a decrease in total urinary salicylates in adult female rats but these

changes were not significant enough to explain the observed increase in the plasma $t_{\frac{1}{2}}$ of salicylate in these animals. However, the estimation of total glucuronides in these studies was based on hydrolysis of urinary samples with β-glucuronidase. Because ester glucuronides of carboxylic acid tend to resist such hydrolysis because of intramolecular rearrangement (Illing & Wilson, 1981; Sinclair & Caldwell, 1982), it is possible that we underestimated salicyl acyl glucuronides and in turn total urinary salicylates in the present studies.

Ovariectomy significantly decreased the plasma $t_{\frac{1}{2}}$ of salicylate and concomitant administration of testosterone exerted no significant effects (Table 5). It therefore appears that the increased $t_{\frac{1}{2}}$ of salicylate in female rats is not due to insignificant levels of testosterone but due to the presence of female sex hormones. Inhibition of metabolism of certain drugs by progesterogens (Juchau & Fouts, 1966; Teunissen *et al.*, 1982) and oestrogens (Kato & Onoda, 1970) has been previously reported.

We have previously shown that PCM reduced the plasma $t_{\frac{1}{2}}$ and increased the Cl_p of salicylate in male rats (comparable to young rats used in the present study) by increasing its metabolism and urinary excretion (Yue & Varma, 1982). The present study shows that a similar influence of PCM on salicylate pharmacokinetics is also exerted in female rats and in older animals. It might be supposed that the mechanism for this change in all different groups of rats is the same as mentioned above for male rats (Yue & Varma, 1982). Kato & Gillette (1965) demonstrated that starvation exerted a stimulatory effect on sex-independent drug metabolizing enzymes in both female and male rats but only on sex-dependent drug metabolizing enzymes in female rats. The present study shows that the plasma half-life of salicylate is influenced by sex and yet at the same time PCM can decrease it in both female and male rats. Whether this is a reflection of substrate selectivity or of the differences between starvation and protein-calorie malnutrition remains unclear.

The age-related changes in the pharmacokinetics of salicylate in male rats seem unrelated to testosterone levels or serum protein-salicylate binding. For instance, testosterone levels were lowest in pups and weanling rats, confirming other reports (Scheer & Robaire, 1980), but the pharmacokinetics of salicylate in these two groups of rats reflected two extremes. Moreover orchidectomy did not change the plasma $t_{\frac{1}{2}}$ of salicylate and testosterone levels in 56–60 week old male rats were insignificantly lower than in young and adult male rats. Although 56–60 week old rats are not senescent, the increase in salicylate plasma $t_{\frac{1}{2}}$ in these animals might be treated as a linear expression of age-related changes, which have been reported for man (Cuny *et al.*, 1979; Montgomery & Sitar, 1981). Kato *et al.* (1964) demonstrated a gradual decrease in the metabolism of drugs from approximately 4 to 35 weeks of age. The old rats used by us were 56–60 weeks of age.

Taking the results of this study as a whole, it would appear that different factors are responsible for changes in the pharmacokinetics of salicylate in different physiological states. Whatever the mechanism for these changes are, in so far as the rat model reflects the human situation, the present studies point to various commonly encountered variables which must be taken into account for a rational salicylate therapy.

We wish to thank Ms Christine Schwenter and Ms Marie Montambault for their technical assistance. This work was supported by a grant from the Quebec Heart Foundation. T.L.Y. is a recipient of a Studentship from the Medical Research Council of Canada.

References

CUNY, G., ROYER, R.J., MUR, J.M., SEROT, J.M., FAURE, G., NETTER, P., MAILLARD, A. & PENIN, F. (1979). Pharmacokinetics of salicylates in elderly. *Gerontology*, **25**, 49–55.

DAVIS, L.E., WESTFALL, B.A. & SHORT, C.R. (1973). Biotransformation and pharmacokinetics of salicylate in newborn animals. *Am. J. Vet. Res.*, **34**, 1105–1108.

FLOWER, R.J., MONCADA, S. & VANE, J.R. (1980). Analgesic-antipyretics and anti-inflammatory agents; drugs employed in the treatment of gout. In *The Pharmacological Basis of Therapeutics*. ed. Gilman, A.G., Goodman, L. & Gilman, A. pp. 682–728. New York: Macmillan.

GARRETTSON, L.K., PROCKNAL, J.A. & LEVY, G. (1975). Fetal acquisition and neonatal elimination of a large amount of salicylate. *Clin. Pharmac. Ther.*, **17**, 98–103.

GIUDICELLI, J.F. & TILLEMENT, J.P. (1977). Influence of sex on drug kinetics in man. *Clin. Pharmacokinet.*, **2**, 157–166.

HUTT, A.J., CALDWELL, J. & SMITH, R.L. (1982). The metabolism of [*carboxyl*-^{14}C]aspirin in man. *Xenobiotica*, **12**, 601–610.

ILLING, H.P.A. & WILSON, I.D. (1981). pH dependent formation of β-glucuronidase resistant conjugates from the biosynthetic ester glucuronide of isoxepac. *Biochem. Pharmac.*, **30**, 3381–3384.

JUCHAU, M.R. & FOUTS, J.R. (1966). Effects of norethynodrel and progesterone on hepatic microsomal drug-metabolizing enzyme systems. *Biochem. Pharmac.*, **15**, 891–898.

KATO, R. & GILLETTE, J.R. (1965). Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. *J. Pharmac. exp. Ther.*, **150**, 279–284.

KATO, R. & ONODA, K. (1970). Studies on the regulation of the activity of drug oxidation in rat liver microsomes by androgen and estrogen. *Biochem. Pharmac.*, **19,** 1649–1660.

KATO, R., VASSANELLI, P., FRONTINO, G. & CHIESARA, E. (1964). Variation in the activity of liver microsomal drug-metabolizing enzymes in rats in relation to the age. *Biochem. Pharmac.*, **13,** 1037–1051.

KRAUER, B. & KRAUER, F. (1977). Drug kinetics in pregnancy. *Clin. Pharmacokinet.*, **2,** 167–181.

KRISHNASWAMY, K. (1978). Drug metabolism and pharmacokinetics in malnutrition. *Clin. Pharmacokinet.*, **3,** 216–240.

LEVY, G. (1979). Pharmacokinetics of salicylate in man. *Drug Metab. Rev.*, **9,** 3–19.

LEVY, G & GARRETTSON, L.K. (1974). Kinetics of salicylate elimination by newborn infants of mothers who ingested aspirin before delivery. *Pediatrics*, **53,** 201–210.

LOWRY, O.H., ROSEBROUGH, H.H., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with Folin phenol reagent. *J. biol. Chem.*, **193,** 265–275.

MENGUY, R., DESBAILLETS, L., MASTERS, Y.F. & OKABE, S. (1971). Evidence for a sex-linked difference in aspirin metabolism. *Nature*, **239,** 102–103.

MONTGOMERY, P.R. & SITAR, D.S. (1981). Increased serum salicylate metabolites with age in patients receiving chronic acetylsalicylic acid therapy. *Gerontology*, **27,** 329–333.

NELSON, E., HANANO, M. & LEVY, G. (1966). Comparative pharmacokinetics of salicylate elimination in man and rats. *J. Pharmac. exp. Ther.*, **153,** 159–166.

O'MALLEY, K., CROOKS, J., DUKE, E. & STEVENSON. I.H. (1971). Effect of age and sex on human drug metabolism. *Br. med. J.*, **3,** 607–609.

PENG, G.W., GADALLA, M.A.F., SMITH, V., PENG, A. & CHIOU, W.L. (1978). Simple and rapid high-pressure liquid chromatographic simultaneous determination of aspirin, salicylic acid, and salicyluric acid in plasma. *J. Pharm. Sci.*, **67,** 710–712.

RAINSFORD, K.D., FORD, N.L.V., BROOKS, P.M. & WATSON, H.M. (1980). Plasma aspirin esterases in normal individuals, patients with alcoholic liver disease and rheumatoid arthritis: characterization and the importance of the enzymic components. *Eur. J. clin. Invest.*, **10,** 413–420.

ROBAIRE, B., EWING, L.L., IRBY, D.C. & DESJARDINS, C. (1979). Interactions of testosterone and estradiol-17β on the reproductive tract of the male rat. *Biol. Reprod.*, **21,** 455–463.

SCHEER, H. & ROBAIRE, B. (1980). Steroid Δ^4-5α-reductase and 3α-hydroxysteroid dehydrogenase in the rat epididymis during development. *Endocrinology*, **107,** 948–953.

SCHMUCKER, D.L. (1979). Age-related changes in drug disposition. *Pharmac. Rev.*, **30,** 445–456.

SINCLAIR, K.A. & CALDWELL, J. (1982). The formation of β-glucuronidase resistant glucuronides by the intramolecular rearrangement of glucuronic acid conjugates at mild alkaline pH. *Biochem. Pharmac.*, **31,** 953–957.

TEUNISSEN, M.W.E., SRIVASTAVA, A.K. & BREIMER, D.D. (1982). Influence of sex and oral contraceptive steroids on antipyrine metabolite formation. *Clin. Pharmac. Ther.*, **32,** 240–246.

VARMA, D.R. (1979). Influence of dietary protein on the anti-inflammatory and ulcerogenic effects and on the pharmacokinetics of phenylbutazone in rats. *J. Pharmac. exp. Ther.*, **211,** 338–344.

VARMA, D.R. (1981). Protein deficiency and drug interactions. *Drug Develop. Res.*, **1,** 183–198.

VARMA, D.R. & YUE, T.L. (1983). The influence of maternal protein deficiency on the placental transfer of salicylate in rats. *Br. J. Pharmac.*, **78,** 233–238.

YUE, T.L. & VARMA, D.R. (1982). Pharmacokinetics, metabolism and disposition of salicylate in protein-deficient rats. *Drug Metab. Dispos.*, **10,** 147–152.

(*Received October 25, 1983.*
Revised December 9, 1983.)