Br. J. Pharmac. (1973), **47**, 69–76.

Effect of 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) on sulphacetamide distribution and excretion in rats

C. MARCHAND AND D. NADEAU

Département de pharmacologie, Faculté de médecine, Université de Montréal, Qué., Canada

Summary

1. Administration of 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A), 40 mg/kg, i.p., simultaneously or 40 min before sulphacetamide sodium, 100 mg/kg, i.p., was associated with a three-fold increase in sulphacetamide plasma concentration of rats. This effect was no longer evident after 30 minutes.

2. The augmentation in sulphacetamide plasma concentration was associated with parallel increases in the muscle, kidney and brain tissue. The stomach was the only organ that contained less sulphacetamide.

3. When sulphacetamide was administered i.v., a similar phenomenon was observed but the differences were less marked.

4. Pretreatment with SKF 525-A was associated with decreased excretion of sulphacetamide by the kidney.

5. It is concluded that SKF 525-A may alter the distribution and excretion of drugs as well as inhibiting drug metabolizing enzymes.

Introduction

Recently, it was shown that pretreatment with 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525-A) changed the tissue distribution of sulphacetamide, *p*-aminosalicylic acid, carbon tetrachloride and barbitone when the compounds were administered orally (Marchand, McLean & Plaa, 1970; McLean & Marchand, 1970; Marchand, McLean, Plaa & Traiger, 1971). Indirect evidence suggested that the effect of SKF 525-A might have been due to inhibition of the gastrointestinal absorption of these compounds. It was then of interest to investigate the effect of SKF 525-A on drugs administered parenterally since it is the most commonly used route of administration of drugs in rats.

Methods

Throughout these experiments, Sprague-Dawley male rats weighing between 150 and 175 g were used. Between 16 and 18 h before each experiment, food was withdrawn but the animals had water *ad libitum*.

Study of sulphacetamide distribution

Rats were pretreated with SKF 525-A, 40 mg/kg, i.p. At different times after pretreatment, the animals were given sulphacetamide sodium, 100 mg/kg, either

through a tail vein or intraperitoneally in a volume of 1·0 ml/kg. Control rats received 0·9% w/v NaCl (saline), i.p., 1·0 ml/kg. Rats were decapitated at different times after administration of sulphacetamide and the blood collected in heparinized tubes. In some experiments, samples of the following tissues were taken: liver (1·0–1·5 g), striated muscle from hind leg (0·4–0·5 g), fat (0·5–0·8 g) from the epididymal region, one kidney and the whole brain. The tissue was then homogenized (Polytron 10) in a solution of 5% trichloroacetic acid. Determination of sulphacetamide was made on clear supernatants after centrifugation at 2,500 rpm for 10 minutes. The method used for determination of sulphacetamide was that of Bratton & Marshall (1939), as modified by Way, Smith, Howie, Weiss & Swanson (1948).

Sulphacetamide of the gastrointestinal tract

Rats were given SKF 525-A, 40 mg/kg, i.p., and sulphacetamide sodium, 100 mg/kg, i.p. Fifteen minutes later, the animals were killed by decapitation. Ligatures were made at the distal oesophagus, the pylorus and the ileocaecal valve. After resection of the stomach and the intestine, the serous side of the tissue was thoroughly washed with saline. The contents of the stomach and the intestine were also washed with 8 and 30 ml of saline respectively. The tissue was then blotted on filter paper, weighed and homogenized with a solution of 5% trichloroacetic acid. Sulphacetamide was determined as previously described.

Urinary excretion

The bladders of rats were surgically exteriorized under ether anaesthesia according to the method of Czaczkes, Kleeman & Koenig (1964). Forty-eight hours after surgery, the rats deprived of food for 16 h were housed in metabolism cages in groups of 4 and were given, simultaneously, SKF 525-A, 40 mg/kg, i.p., and sulphacetamide sodium, 100 mg/kg, i.p. Immediately after this treatment, the animals received, by gavage, a volume of tap water equivalent to 5% of their body weight. The urine was collected, in tubes graduated to 0·1 ml, over a period of 3 h divided as follows: 0–45 min, 45–60 min, 60–120 min and 120–180 minutes. The samples were then frozen for later determination of sulphacetamide.

In order to evaluate the possible effect of SKF 525-A on sulphacetamide conjugation, samples of urine diluted with hydrochloric acid 4 N were hydrolyzed in boiling water for 40 minutes. No detectable amount of conjugated sulphacetamide was found in the urine.

Statistical analysis

Significance of the difference between control and treated rats was assessed by the *t*-test and a P value of 0·05 or less was considered significant.

Results

As illustrated in Figure 1, the plasma of rats that received SKF 525-A and sulphacetamide simultaneously, i.p., contained more sulphacetamide during the first 20 min than did the plasma of the controls that received sulphacetamide but not SKF 525-A. It is interesting that in the control rats the concentration of sulphacetamide remained relatively constant for the first 30 min and then declined for the next

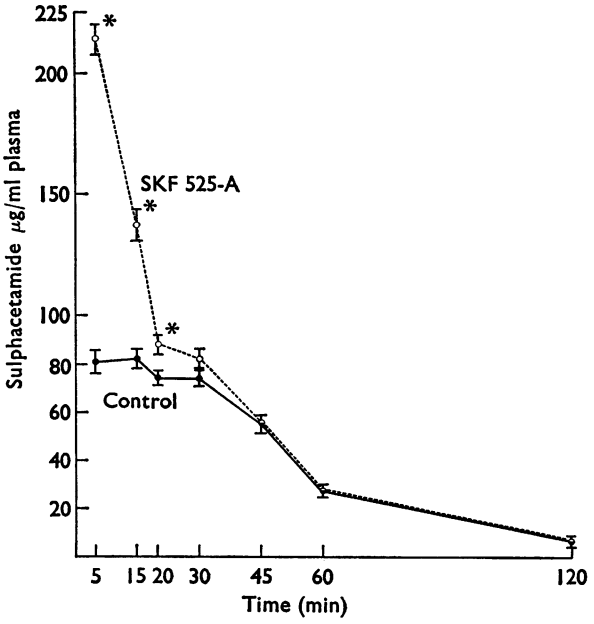

FIG. 1. Plasma disappearance of sulphacetamide sodium, 100 mg/kg, administered intraperitoneally, simultaneously with SKF 525-A, i.p. Each point represents the mean of 6 rats ±S.E.M. * $P<0.05$.

90 minutes. During this latter period, the plasma sulphacetamide concentrations in both the control and SKF 525-A groups were virtually identical. As shown in Table 1, increases in tissue concentrations of sulphacetamide similar to that of plasma were seen in muscle, kidney and brain when the rats were killed 15 min after the i.p. administration of sulphacetamide and SKF 525-A. Sixty minutes after treatment, no differences in the tissue levels of sulphacetamide in the control and SKF 525-A groups were observed, except in muscle where a slightly higher concentration of sulphacetamide was seen in the SKF 525-A treated animals.

Since the effects of SKF 525-A on drug metabolizing enzymes are usually observed 40 min after treatment rather than after simultaneous administration, as in kinetic studies, another experiment was performed in which the sulphacetamide was given i.p., 40 min after the i.p. injection of SKF 525-A or saline. The rats were killed 10 min after sulphacetamide administration. The results (Table 2) indicate that SKF 525-A produced increased concentrations of sulphacetamide in plasma liver, kidney and brain. These increases were quantitatively similar to those seen when SKF 525-A and sulphacetamide were given simultaneously (Table 1).

TABLE 1. *Effect of SKF 525-A, 40 mg/kg, i.p., on tissue distribution of sulphacetamide sodium 100 mg/kg, administered simultaneously by the intraperitoneal route*

Treatment	Time of killing after sulphacetamide injection (min)	Sulphacetamide, μg/g or ml ±S.E.M. Plasma	Liver	Muscle	Kidney	Brain
Saline (7)	15	82·3±3·9	76·1± 7·2	18·2±2·0	341·5±29·6	1·5±0·3
SKF 525-A (6)		137·1±6·4*	79·8±11·2	29·4±3·4*	699·8±99·6*	3·3±0·5*
Saline (7)	60	26·9±2·3	14·6± 1·2	10·1±0·7	99·2±10·7	1·0±0·2
SKF 525-A (7)		28·2±2·6	14·7± 2·0	14·4±1·0*	101·4± 9·7	1·1±0·2

Numbers in parentheses refer to the number of rats used in each experiment. * $P<0.05$.

When the sulphacetamide was given i.v. instead of i.p., the effects of pretreatment with SKF 525-A were somewhat different. Figure 2 illustrates the results of an experiment in which rats were given SKF 525-A or saline, i.p., 40 min before the i.v. administration of sulphacetamide. Consistently higher plasma levels of sulphacetamide were observed in the SKF 525-A group throughout the 40 min observation period. This effect of SKF 525-A was also seen in some other tissues. Significant increases in the levels of sulphacetamide were seen in the plasma, liver and fat of the SKF 525-A-treated animals killed 10 min after sulphacetamide injection (Table 3).

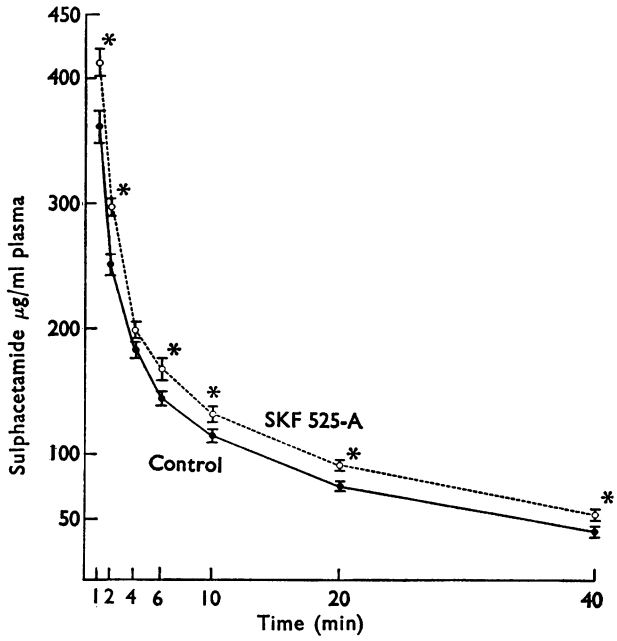

FIG. 2. Effect of 40 min pretreatment with SKF 525-A, 40 mg/kg, i.p., on the plasma disappearance of sulphacetamide, 100 mg/kg, administered intravenously. Each point represents the mean of 6 rats ± S.E.M. *$P<0.05$.

TABLE 2. *Effect of a 40-min pretreatment with SKF 525-A, 40 mg/kg, i.p., on tissue distribution of sulphacetamide sodium, 100 mg/kg i.p. in rats killed 10 min after its injection*

		Sulphacetamide, μg/g or ml ± S.E.M.				
		Plasma	Liver	Muscle	Kidney	Brain
Saline	(7)	93·0±5·1	57·5±4·6	40·7±2·8	341·1±20·0	1·2±0·1
SKF 525-A	(7)	161·6±3·7*	93·6±2·7*	39·1±3·0	677·9±35·1*	2·0±0·0*

Numbers in parentheses refer to the number of rats used in each experiment. * $P<0.05$.

TABLE 3. *Effect of a 40-min pretreatment with SKF 525-A, 40 mg/kg, i.p., on tissue distribution of sulphacetamide sodium, 100 mg/kg*

		Sulphacetamide, μg/g or ml ± S.E.M.				
		Plasma	Liver	Muscle	Fat	Brain
Saline	(7)	115·6±3·3	45·2±0·8	35·3±1·3	19·5±2·4	2·0±0·2
SKF 525-A	(7)	131·8±5·0*	53·1±2·3*	36·8±1·4	43·0±5·7*	2·3±0·1

Numbers in parentheses refer to the number of rats used in each experiment. Rats were killed 10 min after i.v. injection of sulphacetamide. * $P<0.05$.

Increases were also seen in muscle and brain, but these were not statistically significant. The increase in sulphacetamide concentration was also observed when SKF 525-A was given simultaneously; 10 min after i.v. injection, sulphacetamide concentrations were $114{\cdot}6 \pm 4{\cdot}5$ μg/ml in controls and $128{\cdot}6 \pm 1{\cdot}3$ μg/ml in the plasma of rats treated with SKF 525-A ($P<0{\cdot}05$).

Since an increase in tissue concentration of sulphacetamide in rats pretreated with SKF 525-A could be explained by a decrease in sulphacetamide elimination by the kidney, sulphacetamide excretion was studied. As illustrated in Fig. 3, hydrated rats which received SKF 525-A, 40 mg/kg, i.p., and sulphacetamide, 100 mg/kg, i.p. simultaneously, excreted much less urine and sulphacetamide than rats pretreated with saline.

Since it has been shown that sulphacetamide is readily secreted by the stomach (Cooke, Davenport & Goodman, 1941), a possible explanation for the increase in sulphacetamide concentration in tissues of rats pretreated with SKF 525-A would be that SKF 525-A blocks the passage of sulphacetamide from the blood compartment to the stomach. As shown in Table 4, there was much less sulphacetamide

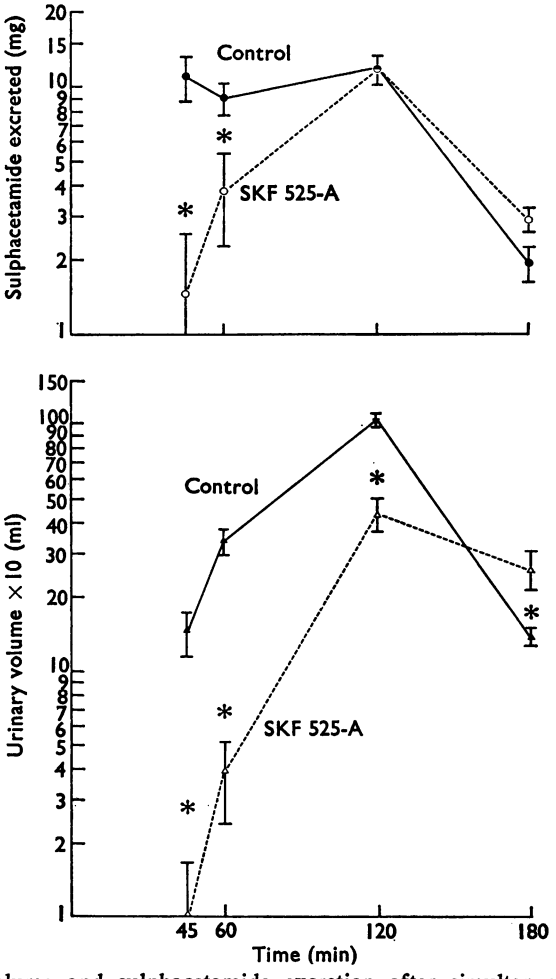

FIG. 3. Urinary volume and sulphacetamide excretion after simultaneous i.p. injection of SKF 525-A, 40 mg/kg and sulphacetamide sodium 100 mg/kg. Each point represents the mean value of 6 groups of rats (4 rats in each group) $\pm$ S.E.M. *$P<0{\cdot}05$.

TABLE 4. *Sulphacetamide in the tissue and the lumen of the gastrointestinal tract in rats killed 15 min after i.p. injection*

Treatment	Plasma	Sulphacetamide Stomach		Small intestine	
		Lumen	Organ	Lumen	Organ
	μg/ml±S.E.M.	μg±S.E.M.	μg±S.E.M.	μg±S.E.M.	μg±S.E.M.
Saline (7)	78·6±2·2	5·6±0·9	72·2±5·2	21·6±1·9	180·3±3·8
SKF 525-A (7)*	138·6±5·1†	4·1±0·8	50·2±2·8†	45·9±5·0†	174·0±8·2

Numbers in parentheses represent the number of rats used in each experiment. * SKF 525-A, 40 mg/kg administered i.p. simultaneously with sulphacetamide sodium, 100 mg/kg. † $P<0·05$.

found in the stomach wall of rats pretreated with SKF 525-A than in controls. However, there was no statistical difference between the amount in the lumen of the stomach of control rats and those pretreated with SKF 525-A. Although there was no difference in the amount of sulphacetamide measured in the intestine wall, the amount found in the lumen of the intestine was higher in rats pretreated with SKF 525-A than in controls.

Discussion

The increase in sulphacetamide tissue concentration in rats treated with SKF 525-A could be due to inhibition of drug metabolizing enzymes in the liver (Mitchell, Reid, Christie, Moskowitz, Krishna & Brodie, 1971). Although such a possibility cannot be completely ruled out, there is evidence that sulphacetamide is not metabolized to a very great extent in the rat. In a study of sulphonamide excretion by the bile duct, Millburn, Smith & Williams (1967) found that sulphacetamide was excreted unchanged in the bile of rats. In the present studies, no conjugated sulphacetamide was detected in the urine of control animals or rats treated with SKF 525-A. Finally, the inhibitory effect of SKF 525-A on drug metabolizing enzymes may be detected up to 10 h after its administration (Cook, Toner & Fellows, 1954) whereas the kinetics studies on the disappearance of sulphacetamide from the plasma indicate that the effect of SKF 525-A has almost disappeared within 1 hour.

Decreased excretion of sulphacetamide by the kidney could be responsible for the augmentation of sulphacetamide in the different tissues of rats pretreated with SKF 525-A. There is evidence that SKF 525-A can affect the kidney functions (Brody, Lukensmeyer & Williamson, 1964; Marshall & Williamson, 1964). Furthermore, Hakim & Fujimoto (1971) have shown that, in chicken, SKF 525-A inhibits tubular transport of morphine. The experiments reported here cannot be used to evaluate this hypothesis. The rats used to study excretion of sulphacetamide had to be hydrated to obtain a sufficient volume of urine in the first hour after injection of sulphacetamide; however, the state of hydration of rats may influence the effect of drugs on kidney functions (Marchand, 1970). This last factor may be important because of the antidiuretic properties of SKF 525-A observed in our experimental conditions and previously reported (Arima & Kuriaki, 1959; Magus, Rickert & Fouts, 1968).

The marked decrease of sulphacetamide in the stomach tissue of rats pretreated with SKF 525-A is compatible with decreased blood flow to the stomach following

SKF 525-A administration (Marchand & Brodeur, 1970; Brodeur & Marchand, 1971). If there is decreased blood flow to the gastrointestinal tract, after administration of SKF 525-A, it is expected that, all other factors being equal, less sulphacetamide will be absorbed from the gastrointestinal tract (Ther & Winne, 1971). On the other hand, if sulphacetamide is administered parenterally, less sulphacetamide will be brought to the stomach with subsequent decreased diffusion of sulphacetamide.

The relatively large quantities of sulphacetamide found in the gastrointestinal tract after parenteral administration in our experiments, are not surprising since Cooke *et al.* (1941) and Davenport (1942) made similar observations in the dog. This passage of sulphacetamide from the vascular compartment to the gastrointestinal tract may be responsible for the plateau in sulphacetamide concentration of the plasma during the first 30 min following i.p. administration of the drug. As to the constant increase in sulphacetamide plasma concentration in rats pretreated with SKF 525-A, after intravenous administration of sulphacetamide, it is compatible with change in the volume of distribution of sulphacetamide. The relatively large quantities of sulphacetamide found in the intestine after parenteral administration could be explained on the basis of the pH partition coefficient which would favour the passage of sulphacetamide (pKa 5·4) from the vascular compartment to the intestine (Shore, Brodie & Hogben, 1957). However, no explanation is readily apparent for the greater quantity of sulphacetamide observed in the intestine of rats treated with SKF 525-A.

There are other mechanisms, not investigated here, which could be responsible for the increase in tissue concentration of sulphacetamide after treatment with SKF 525-A. One of these is the possible diminution of drug excretion by the bile duct. This is an unlikely possibility: a much greater quantity of sulphacetamide was found in the small intestine of rats treated with SKF 525-A than in control animals; SKF 525-A has no apparent effect on the bile flow (Marchand & Brodeur, 1970; Levine, 1970). It is evident that we have not ruled out tissue redistribution of sulphacetamide in rats pretreated with SKF 525-A; it is possible that there are other organs in which sulphacetamide was not measured and which like the stomach contain less sulphacetamide in rats pretreated with SKF 525-A than in control animals.

This work raises the question of the relationship between the tissue concentration of a drug and its pharmacological effect. Since SKF 525-A is known to inhibit drug metabolizing enzymes associated with changes in the pharmacological effects of these drugs, as exemplified by the prolongation of hexobarbitone sleeping time following inhibition of its metabolism (Cook *et al.*, 1954; Axelrod, Reichenthal & Brodie, 1954), it seems that the increased drug concentrations in different tissues of rats treated with SKF 525-A may not play a determining role in the pharmacological effect of this drug. Although the effect of SKF 525-A on drug metabolism may be of primary importance in its ability to prolong hexobarbitone sleeping time, the effect of SKF 525-A on drug distribution may play a leading role in some other effects of SKF 525-A. For example, it may be an explanation for the potentiating effect of SKF 525-A on hypotensive drugs (Goldstein & Rossi, 1959). Furthermore, we have recently shown (unpublished observations) that following administration of barbitone i.p., a drug which is excreted almost intact in the urine (Williams, 1959), the sleep induction time is shorter in rats treated with

SKF 525-A than in control animals. This may be an indication that changes in drug distribution caused by SKF 525-A have pharmacological implications.

This research was supported by the Medical Research Council of Canada. We wish to thank Smith, Kline & French of Canada for their generous supply of SKF 525-A. The authors are grateful to Miss Claudette Lamoureux for her technical assistance.

REFERENCES

ARIMA, K. & KURIAKI, K. (1959). Some pharmacological studies on the renal carbonic anhydrase activity and electrolytes excretion in the rat. *Jap. J. Pharmac.*, **8**, 165–169.

AXELROD, J., REICHENTHAL, J. & BRODIE, B. B. (1954). Mechanism of the potentiating action of β-diethylaminoethyl diphenylpropyl acetate. *J. Pharmac. exp. Ther.*, **112**, 49–54.

BRATTON, A. C. & MARSHALL, E. K. (1939). A new coupling component for sulfanilamide determination. *J. biol. Chem.*, **128**, 537–550.

BRODEUR, J. & MARCHAND, C. (1971). Effect of splenectomy on the activity of drug-metabolizing enzymes in the liver of rats. *Can. J. Physiol. Pharmac.*, **49**, 161–166.

BRODY, M. J., LUKENSMEYER, W. A. & WILLIAMSON, H. E. (1964). Inhibition of autoregulation of renal blood flow by SKF 525-A and fluoroacetate. *Proc. Soc. exp. Biol.*, **117**, 79–83.

COOK, L., TONER, J. J. & FELLOWS, E. J. (1954). The effect of β-diethylaminoethyldiphenylpropyl-acetate hydrochloride (SKF No. 525-A) on hexobarbital. *J. Pharmac. exp. Ther.*, **111**, 131–141.

COOKE, M., DAVENPORT, H. W. & GOODMAN, L. S. (1941). The secretion of sulfonamide drugs in gastric juice. *Yale J. biol. Med.*, **14**, 13–28.

CZACZKES, J. W., KLEEMAN, C. R. & KOENIG, M. (1964). Physiologic studies of antidiuretic hormone by its direct measurement in human plasma. *J. clin. Invest.*, **43**, 1625–1640.

DAVENPORT, H. W. (1942). The mechanism of the secretion of sulfonamide drugs in gastric juice. *Yale J. biol. Med.*, **14**, 589–597.

GOLDSTEIN, S. & ROSSI, G. V. (1959). Potentiation of the activity of hypotensive drugs: effect of several synthetic compounds on six hypotensive agents in hypertensive rats and normotensive dogs. *J. Pharmac. exp. Ther.*, **147**, 409–415.

HAKIM, R. & FUJIMOTO, J. M. (1971). Inhibition of renal tubular transport of morphine by β-diethylaminoethyl diphenylpropylacetate in the chicken. *Biochem. Pharmac.*, **20**, 2647–2662.

LEVINE, W. G. (1970). Effect of SKF 525-A on biliary function. Influence of body temperature. *Life Sci.*, **9**, Part 1, 437–442.

MAGUS, R. D., RICKERT, D. E. & FOUTS, J. R. (1968). Activation of hydrocortisone-induced tryptophan pyrrolase of rat liver by SKF 525-A. The possible involvement of antidiuric hormone. *Biochem. Pharmac.*, **17**, 2071–2080.

MARCHAND, C. (1970). The diuretic and antidiuretic effect of morphine sulphate in rats. *Proc. Soc. exp. Biol.*, **133**, 1303–1306.

MARCHAND, C. & BRODEUR, J. (1970). Change in colloidal gold clearance in rats treated with SKF 525-A. *Rev. canad. Biol.*, **29**, 293–298.

MARCHAND, C., MCLEAN, S. & PLAA, G. L. (1970). The effect of SKF 525-A on the distribution of carbon tetrachloride in rats. *J. Pharmac. exp. Ther.*, **174**, 232–238.

MARCHAND, C., MCLEAN, S., PLAA, G. L. & TRAIGER, G. (1971). Protection by 2-diethyl-aminoethyl-2, 2-diphenylvalerate hydrochloride against carbon tetrachloride hepatotoxicity. A possible mechanism of action. *Biochem. Pharmac.*, **20**, 869–875.

MARSHALL, F. N. & WILLIAMSON, H. E. (1964). Natriuretic response during infusion of β-diethyl-aminoethyldiphenylpropyl acetate hydrochloride (SKF 525-A) into the renal artery. *J. Pharmac. exp. Ther.*, **143**, 395–400.

MCLEAN, S. & MARCHAND, C. (1970). The effect of SKF 525-A on drug concentration in the blood. *Life Sci.*, **9**, Part 1, 1075–1080.

MILLBURN, P., SMITH, R. L. & WILLIAMS, R. T. (1967). Biliary excretion of foreign compounds sulphonamide drugs in the rat. *Biochem. J.*, **105**, 1283–1287.

MITCHELL, J. R., REID, W. D., CHRISTIE, B., MOSKOWITZ, J., KRISHNA, C. & BRODIE, B. B. (1971). Bromobenzene-induced hepatic necrosis: species differences and protection by SKF 525-A. *Res. Com. Chem. Path. Pharmac.*, **2**, 877–888.

SHORE, P. A., BRODIE, B. B. & HOGBEN, C. A. M. (1957). The gastric secretion of drugs: a pH partition hypothesis. *J. Pharmac. exp. Ther.*, **119**, 361–369.

THER, L. & WINNE, D. (1971). Drug absorption. *Ann. Rev. Pharmac.*, **11**, 57–68.

WAY, E. L., SMITH, P. K., HOWIE, D. L., WEISS, R. & SWANSON, R. (1948). The absorption, distribution, excretion and fate of para-aminosalicyclic acid. *J. Pharmac. exp. Ther.*, **93**, 368–382.

WILLIAMS, R. T. (1959). *Detoxication Mechanism*, p. 596. London: Chapman and Hall.

(*Received June* 23, 1972)