be seriously in error. *p*-Aminohippuric acid is used routinely for the evaluation of kidney function and effective renal blood flow. It was noticed that in some species consistently low clearance values were being obtained, and this finding was traced to the metabolism occurring in the kidney (23).

In the clinical situation, it may be important to know what types of drugs are metabolized in the kidney and the extent to which this occurs. One should be aware of the possible effects of administering these drugs to anephric patients or to patients suffering from any form of renal failure. These patients often have other accompanying ailments and may be treated with a large variety of drugs. Accumulation of drugs and toxic reactions may occur if the kidneys no longer contribute to metabolism.

REFERENCES

(1) M. Dixon and E. C. Webb, "Enzymes," Longmans, Green, London, England, 1958.

(2) P. D. Boyer, H. Landy, and K. Myrbäck, "The Enzymes," vol. 4, 2nd ed., Academic, New York, N. Y., and London, England, 1960.

(3) R. T. Williams, "Detoxication Mechanisms," 2nd ed., Wiley, New York, N. Y., 1959.

(4) H. Remmer, *Ann. Rev. Pharmacol.*, **5**, 405(1965).

(5) W. H. Barr and S. Riegelman, *J. Pharm. Sci.*, **59**, 154(1970).

(6) *Ibid.*, **59**, 164(1970).

(7) W. C. Hülsmann and L. W. Statius van Eps, *Clin. Chim. Acta*, **15**, 233(1967).

(8) A. J. Quick, *J. Biol. Chem.*, **96**, 73(1932).

(9) P. K. Knoefel, K. C. Huang, and A. Despopoulos, *Amer. J. Physiol.*, **196**, 1224(1959).

(10) G. Bunge and O. Schmiedeberg, *Arch. Exp. Pathol. Pharmakol.*, **6**, 233(1876–1877).

(11) W. Kochs, *Arch. Ges. Physiol.*, **20**, 64(1874).

(12) I. Snapper, A. Grünbaum, and J. Neuberg, *Biochemistry*, **145**, 10(1924).

(13) H. G. Bray, W. V. Thorpe, and K. White, *Biochem. J.*, **48**, 88(1951).

(14) N. G. Heatley, *Ant. Med. Clin. Ther.*, **2**, 33(1956).

(15) T. R. Tephly, R. E. Parks, Jr., and G. J. Mannering, *J. Pharmacol. Exp. Ther.*, **143**, 292(1964).

(16) C. J. Umberger and F. F. Fiorese, *Clin. Chem.*, **9**, 91(1963).

(17) "Liquid Scintillation Counting," C. G. Bell, Jr., and F. N. Hayes, Eds., Pergamon, New York, N. Y., 1958, p. 88.

(18) B. Josephson, A. Grieg, G. Kakossaios, and J. Kallas, *Acta Physiol. Scand.*, **30**, 11(1953).

(19) B. Woolf, quoted in "Allgemeine Chemie der Enzyme," by J. B. S. Haldane and K. G. Stern, Steinkopf-Verlag, Dresden, Germany, 1932, p. 119.

(20) R. P. Forsyth, A. S. Nies, F. Wyler, J. Neutze, and K. L. Melmon, *J. Appl. Physiol.*, **25**, 736(1968).

(21) A. J. Quick, *J. Biol. Chem.*, **92**, 65(1931).

(22) E. Krüger-Thiemer and R. R. Levine, *Arzneim.-Forsch.*, **18**, 1575(1968).

(23) B. P. Setchell and E. Blanch, *Nature*, **189**, 230(1961).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 14, 1971, from the *Department of Pharmacy, School of Pharmacy, University of California, San Francisco, CA 94122*

Accepted for publication November 18, 1971.

Abstracted from a dissertation submitted by Suk Han Wan to the Graduate Division, University of California, San Francisco, in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by a grant-in-aid from the Academic Senate Committee on Research, San Francisco Division, University of California; a grant-in-aid from the Patent Board, University of California; and Grant NIGMS 16496 from the National Institutes of Health, U. S. Public Health Service, Bethesda, MD 20014

The authors thank Dr. Ralph P. Forsyth for advice and assistance with the monkey used in the study.

* Present address: Department of Clinical Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103

▲ To whom inquiries should be directed.

Renal Contribution to Overall Metabolism of Drugs II: Biotransformation of Salicylic Acid to Salicyluric Acid

SUK HAN WAN* and SIDNEY RIEGELMAN▲

Abstract □ The biotransformation of salicylic acid to salicyluric acid in the Rhesus monkey was studied, and the contributions made by the kidney to the metabolic process were estimated from analysis of clearance data.

Keyphrases □ Salicylic acid biotransformation to salicyluric acid—renal metabolism contribution, renal and apparent clearances, Rhesus monkey □ Salicyluric acid from salicylic acid biotransformation—renal metabolism contribution, renal and apparent clearances, Rhesus monkey □ Renal metabolism—biotransformation of salicylic acid to salicyluric acid, renal and apparent clearances, Rhesus monkey □ Biotransformation kinetics—salicylic acid to salicyluric acid, renal metabolism contribution, Rhesus monkey

It was shown in a previous paper (1) that the conversion of benzoic acid to hippuric acid occurs in the kidney of the rabbit. This paper presents a similar study of salicylic acid metabolism to salicyluric acid in the monkey. After reviewing the literature, it was found that none of the laboratory animals have been reported to metabolize salicylic acid (SA) to salicyluric acid (SAU) in quantities similar to those of man. No information was available on the metabolism of salicylic acid in the Rhesus monkey, a useful experimental animal. A preliminary study was undertaken to determine the fate of salicylic acid in this animal. At a dose equivalent to a 625-mg. dose/70-kg. man, it was found that the Rhesus monkey excreted high amounts of salicyluric

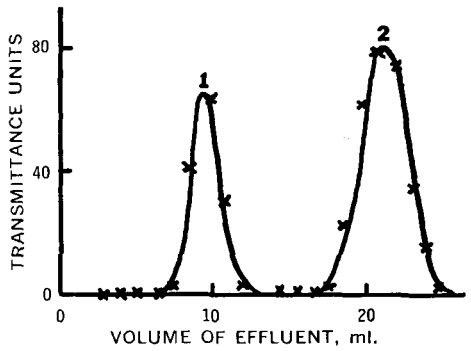

Figure 1—*Elution of salicylic acid (2) and salicyluric acid (1) with phosphate buffer from Column 1, Sephadex G10 (7 mm. o.d. × 120 mm.).*

acid. As noted later, 72% of the administered dose was recovered as salicyluric acid, while man produces 50–80% (2). It was, therefore, decided to use the Rhesus monkey as a test animal for the study of conversion of salicylic acid to salicyluric acid.

EXPERIMENTAL

Materials—Salicylic acid carboxy-^{14}C, which had a specific activity of 4.6 mc./m*M*, was used for the synthesis of salicyluric acid carboxy-^{14}C. The procedure used was similar to that described by Frömming and Vollenberg (3).

Animal Preparation—Catheters were implanted in the aorta, inferior vena cava, and both ureters of a male Rhesus monkey weighing 5.6 kg.

Intravenous Study—A sterile solution of 57.97 mg. sodium salicylate, equivalent to 50 mg. salicylic acid, was administered *via* the inferior vena cava catheter. Blood samples of 2.5 ml. were taken from the aorta catheter at 4, 8, 12, 16, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, and 300 min. Total urine was collected over 16 hr. The plasma samples were extracted with ether and analyzed fluorimetrically for salicylic acid (4). Salicyluric acid in urine was analyzed colorimetrically using the method described by Umberger and Fiorese (5).

Infusion Study—Radiotagged salicyluric acid, specific activity 0.053 μc./m*M*, was infused at a rate of 30 mg./hr., preceded by a priming dose of 10 mg. Infusion of salicyluric acid was maintained at this rate throughout the entire experiment. Four blood and five urine samples were taken during the 2nd hr. of infusion when steady state was presumed to have been reached. The sodium salicylate infusion solution was made by dissolving 400 mg. salicylic acid equivalent in 100 ml. saline. The infusion rate of this solution ranged from 3.6 to 18 mg./hr. The initial infusion was preceded by a priming dose of 10 mg. salicylic acid. Infusion of salicylic acid was started 60 min. after the priming dose. At an infusion rate of 3.6 mg./hr., the expected steady-state plasma concentration is 2.5 mcg./ml. The actual experimental value was 2.4 mcg./ml. Blood and urine samples were obtained during the 2nd hr. of infusion at each infusion rate. All solutions to be intravenously administered were prepared in sterile vessels and were bacteriologically filtered.

Analytical Methods—The amounts of salicyluric acid and salicylic acid in plasma are relatively low, in the region of 4–30 mcg./ml. Various methods have been used in the analyses of these compounds together or individually (6–9). Some of these methods are satisfactory for the analysis of large amounts of either compound such as quantitation in urine, but they are inadequate for the more sensitive analyses required for plasma.

Sinha and Gabrielli (10) discussed a successful separation of benzoic acid and hippuric acid on a Sephadex gel column. This method allows one to separate the compounds and avoid partial interference in their later assay. Lee *et al.* (11) showed that it is possible to separate salicylic acid and aspirin on at least two types of Sephadex columns.

Two types of Sephadex, A25 and G10, were tried in an attempt to separate salicylic acid and salicyluric acid. G10 gave better resolution. The procedure for quantitation of salicylic acid and salicyluric acid is as follows. Separation was first effected on a Sephadex G10 column, and the eluate was analyzed by fluorimetry. Sephadex G10, 40–120-μ particle size[1], was washed with pH 7.0 0.067 *M* phosphate buffer to remove unwetted particles and allowed to soak in buffer overnight. The slurry was poured into glass columns[2] (7 mm. i.d.). The column height was adjusted to 120 mm. The column packing was cleaned by eluting with 20–30 ml. buffer. For the elution of samples, the level of buffer in the column was allowed to run to just above the bed surface. Aqueous samples of up to 40 mcg. salicylic acid/salicyluric acid mixture were carefully added to the gel bed surface and allowed to soak through. The volume of sample did not exceed 500 μl. The column was eluted with phosphate buffer, and the eluate was collected in 1-ml. fractions. The 1-ml. eluate was diluted to 5 ml. with buffer and measured fluorometrically[3]. Activation wavelength and emission wavelength were 305 and 400 nm., respectively. The slit width was 6 nm.

The elution pattern of salicylic acid and salicyluric acid on several columns of the same bed volume were similar (Fig. 1). A 5–6-ml. effluent was collected before salicyluric acid was eluted. This volume is greater than the void volume of 1.2 ml., and proteins in samples should be eluted in this first effluent fraction. Salicyluric acid was eluted in the 6–13-ml. fraction. There was no drug in the 13–16-ml. fraction. Salicylic acid was eluted in the 16–27-ml. fraction. The difference of 3 ml. between the two drug fractions is large enough for a clear separation. There was no binding of drug to columns and recovery was complete.

The proteins in plasma samples were precipitated by heating or by adding equal volumes of isopropyl alcohol. A 0.2–0.5-ml. aliquot of the supernate was placed on the column and eluted with buffer. The first 4 ml. of effluent was discarded. The second

Table I—Pharmacokinetic Parameters Obtained after Intravenous Administration of Salicylic Acid to the Monkey[a]

Dose, mg.	*A*, mcg. ml.$^{-1}$	*B*, mcg. ml.$^{-1}$	C_p^0, mcg. ml.$^{-1}$	α, min.$^{-1}$	β, min.$^{-1}$	k_{el}, min.$^{-1}$	V_p, ml.
50	70	46	116	0.043	0.014	0.024	431

[a] Weight of monkey = 5.6 kg.

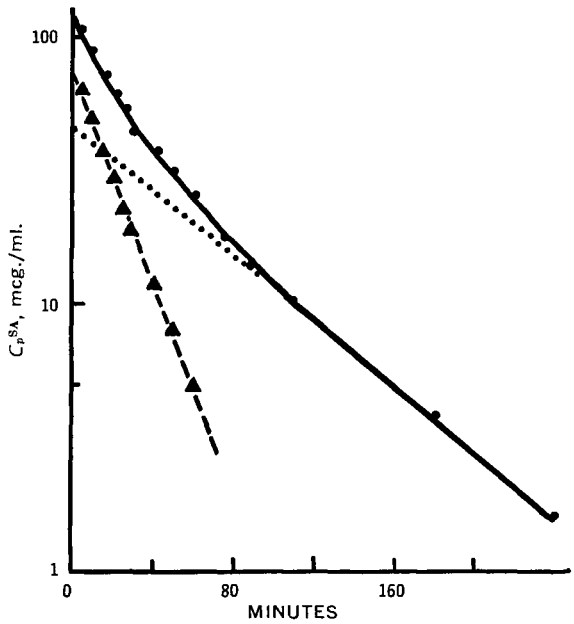

Figure 2—*Plasma concentration–time curve obtained after intravenous administration of 50 mg. salicylic acid to the monkey.*

[1] Pharmacia.
[2] Biorad Laboratories.
[3] Perkin-Elmer MPF-2A fluorescence spectrophotometer.

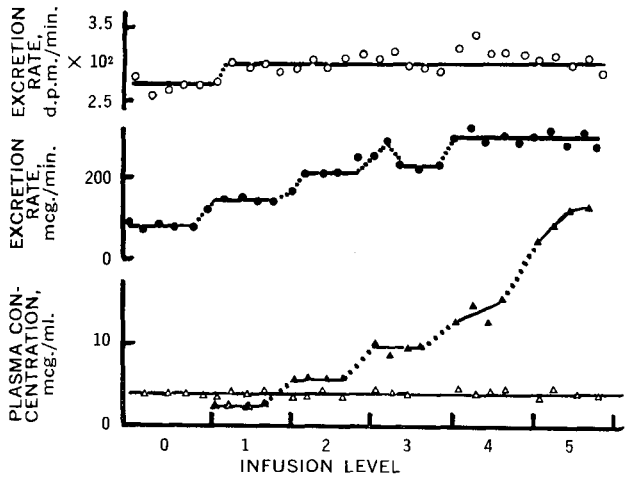

Figure 3—*Plasma concentrations and urinary excretion rates during sequential infusion of salicyluric acid and salicylic acid in the monkey. Key:* ▲, C_p^{SA} (*mcg. ml.*$^{-1}$); △, C_p^{SAU} (*mcg. ml.*$^{-1}$); ●, $\Delta A^{SAU}/\Delta t$ (*mcg. min.*$^{-1}$); *and* ○, $(\Delta A^{SAU*}/\Delta t)$ (*d.p.m. min.*$^{-1}$). *Each solid line and each broken line represent a 1-hr. interval.*

fraction of 10 ml. contains all of the salicyluric acid and the third fraction of 13 ml. contains salicylic acid. Plasma blank samples subjected to this treatment gave high fluorimetric readings only in the first 3 ml. of discard. Urine samples were diluted appropriately and an estimated amount of drug not exceeding 10 mcg. of either compound was added to the column and eluted with buffer. The time of elution was about 2 hr.

RESULTS AND DISCUSSION

The dose of salicylic acid given intravenously to the monkey is equivalent to a 625-mg. dose for a 70-kg. man. The terminal half-life for the animal studied was 50 min. (Fig. 2), which is shorter than the 3–4-hr. average half-life in man. The other pharmacokinetic parameters for this animal have been tabulated in Table I. The clearance value for salicylic acid estimated from the area under the curve was 10 ml./min. or 1.8 ml./min./kg., in marked contrast to the data obtained for benzoic acid in rabbits where clearance was found to be between 44 and 115 ml./min. or 12.6–28.8 ml./min./kg. In a single intravenous study in the monkey, benzoic acid was found to have a plasma clearance of 140 ml./min. or 38.1 ml./min./kg. Plasma clearance of salicylic acid in man, at a dose level of about 500 mg. administered intravenously, was found to be in the range of 0.57 ml./min./kg. body weight according to the data of Riegelman *et al.* (12).

The fraction of the dose excreted as salicyluric acid in 16 hr. was found to be 72%.

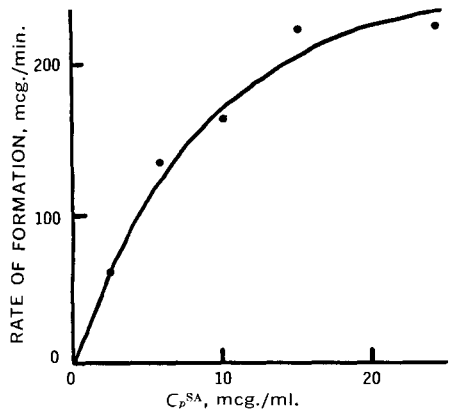

Figure 4—*Rate of metabolism of salicylic acid to salicyluric acid by the kidney at different plasma concentrations of salicylic acid during infusion of salicylic acid in the monkey.*

Table II—Metabolism and Excretion of Salicylic Acid and Specific Organ Contributions to Metabolism at Various Steady-State Plasma Concentrations of Salicylic Acid

C_p^{SA}, mcg. ml.$^{-1}$	Percent SAU —Formed in— Kidney	Liver	Percent Dose Excreted as Salicyluric Acid	Percent Dose Excreted as Salicylic Acid
2.4	~100	0	48.4	4.6
5.7	100	0	65.3	8.0
9.8	100	0	55.5	5.3
13.5	100	0	59.3	19.2
22.5–26.5	100	0	47.4	16.4

Figure 3 shows the plasma levels of salicylic acid and salicyluric acid and rates of excretion of salicyluric acid and ^{14}C-radiotagged salicyluric acid as obtained from the infusion experiment. The rate of excretion of ^{14}C-radioactively labeled salicyluric acid was increased when salicylic acid infusion was begun, but it remained constant thereafter for increasing rates of salicylic acid infusion. The clearance change points out the necessity for monitoring the constancy of the excretory process. The change may have been caused by salicylic acid displacing protein binding or tissue binding of salicyluric acid, possibly increasing the glomerular filtration rate of salicyluric acid. Therefore, the renal clearance for subsequent calculations was corrected for this increase. The clearance of ^{14}C-radioactively labeled salicyluric acid in the monkey was 28 ml./min. or 5 ml./min./kg. This clearance is low compared to a preliminary study in a 14-kg. dog, which gave a clearance value of 146 ml./min., equivalent to 10 ml./min./kg. Unfortunately, no similar experiment was done in this monkey prior to surgery. The renal plasma flow for the monkey was estimated to range from 18 to 21 ml./min./kg. (13), while the *p*-aminohippuric acid clearance in dogs was found to range from 8.7 to 14.9 ml./min./kg. (14).

Discussions of these results with a physiologist led us to postulate that the tubing used to cannulate the ureters was too small in internal diameter, leading to a high resistance in flow. The necessary length of tubing was 5–6 times the length of the ureters in the monkey. This may cause a build-up of fluid in the kidney tubules, resulting in ischemia. With a reduced flow, one would obviously see a reduced renal clearance. It must be emphasized, however, that clearance was constant throughout the experiment, beyond the initial increase already discussed. Further studies will be required to elucidate whether a possible ischemic reaction has a significant influence on these results.

Plasma concentrations of salicylic acid were at steady state during the first three levels of infusion. At the fourth and fifth levels of infusion, the concentrations of salicylic acid did not reach steady state during the 2-hr. infusion period, and the animal may have been approaching saturation of the conjugating enzymes. Changes in plasma concentration of salicyluric acid are not detectable during the entire period of salicylic acid infusion. The assay method used for the plasma salicyluric acid is believed to be accurate to at least 0.5 mcg./ml. of sample. If any salicyluric acid was being formed in the liver or other metabolic sites, an increase should be seen in the plasma level of salicyluric acid above the exogenous steady-state level of salicyluric acid, namely 3.9 mcg./ml. The urine excretion rate of salicyluric acid, however, increases at each rate of salicylic acid infusion except for the fifth infusion level. The results show that liver metabolism is very low and that practically all of the salicyluric acid is being formed in the kidney.

The method for assessment of kidney contribution to overall metabolism was previously discussed (1). The rate of formation of salicyluric acid by all metabolic sites includes the rate of metabolism by all extrarenal sites, presumed to be the liver, and the rate of metabolism occurring in the kidney. The extrarenal contribution can be estimated from the salicyluric acid clearance calculation based on the radioactivity measurement and the plasma steady-state concentrations of salicyluric acid (Eq. 6 of *Reference 1*). The kidney contribution to the salicyluric acid formation is estimated by the difference between the observed rate of total metabolism from the calculated extrarenal contribution (Eq. 7 of *Reference 1*).

Table II shows the percentage of salicylic acid converted to salicyluric acid by the kidney at different plasma levels of salicylic acid. As expected, the fraction of the dose metabolized to salicyluric acid decreased with increasing plasma concentrations of salicylic

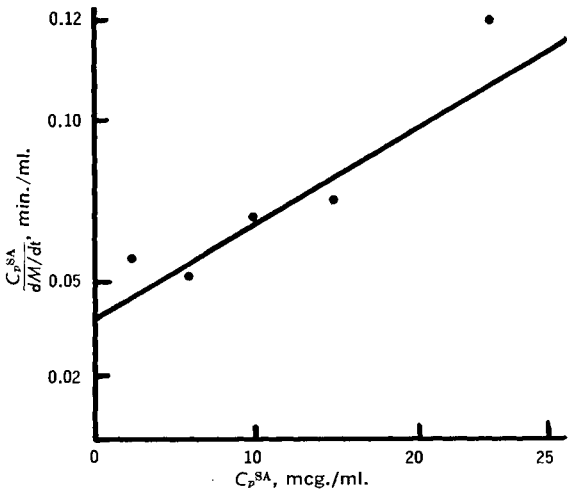

Figure 5—*Woolf plot of* S/V versus S *for enzymic conversion of salicyluric acid from salicylic acid in the monkey.*

acid. At the higher plasma concentrations, a larger fraction of the acid was excreted unchanged.

Figure 4 is a plot of (dM/dt)SAU against C_p^{SA} and is indicative of what one expects of a capacity-limited enzyme system. The Woolf plot (Fig. 5) gave a $V_{max.}$ of 346 mcg./min. and a K_m of 13.4. The correlation coefficient for the regression line was 0.91.

From these data, the conversion of salicylic acid to salicyluric acid seems to occur mainly in the kidney in the monkey. The calculated maximum rate of metabolism to salicyluric acid was lower than for hippuric acid in the rabbit. In most species, the metabolism of benzoic acid proceeds at a rate that is much faster than the rate of conversion of salicylic acid to salicyluric acid. In man, metabolism of benzoic acid proceeds at a rate which is 20-fold that for salicylic acid (15). The capacity-limiting factor in benzoic acid metabolism was shown by Quick (16) and Levy and Matsuzawa (17) to be due to some extent to the availability of glycine. However, saturation of salicylic acid metabolism is probably not due to the same reason. The turnover rate of glycine evoked in the metabolism of salicylic acid is much lower. It has also been shown that when glycine is administered simultaneously with salicylic acid, the maximal rate of formation of salicyluric acid is not changed (18). It is possible that capacity-limited metabolism is therefore due to saturation of an enzyme system rather than to the rate of availability of glycine.

All the results discussed in this report are, of course, dependent on the hemodynamic state of the animal during the experimental study. If it is presumed that blood flow to the kidney was reduced, conditions for saturation may be different from what is found under normal hemodynamic conditions. Moreover, this could allow for a greater fraction of the drug to be metabolized by the liver, a condition that was not observed, since the liver for most animals is known to have a significant concentration of glycine-conjugating enzyme.

REFERENCES

(1) S. H. Wan and S. Riegelman, *J. Pharm. Sci.*, **61**, 1278(1972).

(2) M. J. H. Smith and P. K. Smith, "The Salicylates," Interscience, New York, N. Y., 1966.

(3) K. H. Frömming and W. Vollenberg, *Arch. Pharm.*, **299**, 179(1966).

(4) M. Chirigos and S. Udenfriend, *J. Lab. Clin. Med.*, **54**, 769(1959).

(5) C. J. Umberger and F. F. Fiorese, *Clin. Chem.*, **9**, 91(1963).

(6) R. E. Galloway, H. C. Elliott, and A. A. Walker, III, *Ala. J. Med. Sci.*, **2**, 105(1965).

(7) B. B. Brodie, S. Udenfriend, and A. K. Coburn, *J. Pharmacol. Exp. Ther.*, **80**, 114(1944).

(8) P. K. Smith, H. L. Gleason, C. G. Stoll, and S. Ogorzalet, *ibid.*, **87**, 237(1946).

(9) D. Schacter and J. G. Manis, *J. Clin. Invest.*, **37**, 800(1958).

(10) S. N. Sinha and E. R. Gabrielli, *Clin. Chim. Acta*, **19**, 313(1968).

(11) K. H. Lee, L. Thompkins, and M. R. Spencer, *J. Pharm. Sci.*, **57**, 1240(1968).

(12) S. Riegelman, J. Loo, and M. Rowland, *ibid.*, **57**, 128(1968).

(13) R. P. Forsyth, A. S. Nies, F. Wyler, J. Neutze, and K. L. Melmon, *Appl. Physiol.*, **25**, 736(1968).

(14) M. J. Mandel, D. G. Vidt, and L. A. Sapirstein, *Amer. J. Physiol.*, **182**, 428(1955).

(15) G. Levy, *J. Pharm. Sci.*, **54**, 959(1965).

(16) A. J. Quick, *J. Biol. Chem.*, **92**, 65(1931).

(17) G. Levy and T. J. Matsuzawa, *J. Pharmacol. Exp. Ther.*, **156**, 285(1967).

(18) E. Nelson, M. Hanano, and G. Levy, *ibid.*, **153**, 159(1966).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 7, 1971, from the *Department of Pharmacy, School of Pharmacy, University of California, San Francisco, CA 94122*

Accepted for publication November 18, 1971.

Abstracted from a dissertation submitted by Suk Han Wan to the Graduate Division, University of California, San Francisco, in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by a grant-in-aid from the Academic Senate Committee on Research, San Francisco Division, University of California; a grant-in-aid from the Patent Board, University of California; and Grant NIGMS 16496 from the National Institutes of Health, U. S. Public Health Service, Bethesda, MD 20014

The authors gratefully acknowledge Dr. Ralph P. Forsyth for consultations on surgical procedures on the monkey, Dr. Shaikh M. Matin for the synthesis of radiotagged salicyluric acid, and Dr. Lawrence Earley for helpful discussions.

* Present address: Department of Clinical Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103

▲ To whom inquiries should be directed.