Br. J. Pharmac. (1976), **56**, 423–429

THE DISTRIBUTION AND KINETICS OF SULPHACETAMIDE IN LEUKAEMIC MICE

C. MARCHAND & D. NADEAU

Département de pharmacologie, Faculté de médecine, Université de Montréal, Montréal, Québec, Canada H3C 3J7

1 During the first 90 min following oral administration of sulphacetamide, there was a rapid decline in plasma drug concentration in control mice whereas a progressive increase in sulphacetamide concentration was observed in leukaemic mice.

2 Similar changes in the kinetics of sulphacetamide distribution were observed in the liver, spleen and muscle.

3 While the concentration of sulphacetamide remained quite constant in the brain and fat tissue of control mice, a progressive increase in drug concentration was observed in the brain and fat tissue of leukaemic mice.

4 Some of these changes in the kinetics of sulphacetamide tissue distribution are compatible with delay in gastrointestinal absorption of the drug and its accumulation in the ascitic fluid.

Introduction

It is well established that there is a decrease in drug metabolizing enzyme activity in the liver of tumour-bearing rats (Adamson & Fouts, 1961; Kato, Frontino & Vassanelli, 1963). This is associated with prolongation of drug action when it is administered by the intravenous or intraperitoneal route (Kato, Takanaka & Oshima, 1968; Rosso, Donelli, Franchi & Garattini, 1971).

In recent studies, it was shown that not only drug biotransformation was affected by the disease but tissue distribution kinetics of sulphacetamide were modified in Walker tumour-bearing rats (Nadeau & Marchand, 1975). These changes could be partially explained by delay in gastrointestinal absorption of the drug. Decrease in the rate of drug elimination was also believed to play a role in these changes of distribution kinetics. Since these observations were made with a solid type of tumour, it was of interest to study the effect of leukaemia L-1210 on the distribution of sulphacetamide in another species, the mouse.

Methods

BDF[1] male mice weighing between 18 and 20 g were obtained from Sprague-Dawley, Madison, Wisconsin. The leukaemia strain was obtained through the courtesy of Microbiological Ass., Bethesda, Maryland. The ascitic fluid was aspirated with sterile instruments from the abdominal cavity of acutely ill mice and 0.1 ml of this fluid, containing 10^6 to 10^7 leukocytes was injected into the peritoneal cavity of healthy mice. The survival time of these animals was 6 to 7 days.

Five days after injection of ascitic fluid, animals were starved overnight but allowed water *ad libitum*. Their weights were 18.9 ± 0.3 g and 23.1 ± 0.5 g for control and leukaemic mice respectively. These mice were then given sulphacetamide sodium 200 mg/kg, by the oral route in a volume of 20 ml/kg. At different times after drug administration, the mice were killed by decapitation and the blood collected in heparin-treated tubes. The abdominal cavity was then opened and the ascitic fluid aspirated with a syringe. After ligature of the cardia and the pylorus, the stomach and the intestine were taken out as well as the liver, spleen, kidney, brain, fat tissue (50–400 mg) from the epididymal region, and 250 to 450 mg of striated muscle from the hind legs.

The tissue was then homogenized (Polytron 10) in a 5% solution of trichloroacetic acid. Determination of sulphacetamide was made on clear supernatants after centrifugation at 2,500 rev/min for 10 minutes. Whenever the supernatant was not clear, fat was extracted with chloroform. Determination of sulphacetamide was made by the method of Bratton & Marshall (1939), as modified by Way, Smith, Howie, Weiss & Swanson (1948). Since tissue blanks gave a slight but detectable colouration at 545 nm, readings

were corrected with blanks prepared from tissues of control and leukaemic mice.

Plasma half-life ($T_{½}$) of sulphacetamide was approximated from the regression line obtained from the log of drug plasma concentration *vs* time. This regression line was calculated from individual data and the correlation coefficient (r) estimated. The error variance, the 95% confidence limits for the regression line and the 95% confidence intervals of the slope (β) were calculated as described by Goldstein (1964).

The areas under the curves (AUC) were approximated as sums of trapezoids. Let C_i and C'_i be the mean sulphacetamide concentration in the plasma at time t_i of a group of control mice and of a group of leukaemic animals respectively. Let A and A' be the area under the curve for control and for leukaemic mice respectively. Then, for the control group,

$$A = (t_2 - t_1)(C_1 + C_2)/2 + (t_3 - t_2)(C_2 + C_3)/2 + (t_4 - t_3)(C_3 + C_4)/2 + (t_5 - t_4)(C_4 + C_5)/2$$

which may be written in the form

$$A = \sum_{i=1}^{5} k_i C_i$$

where $k_1 = (t_2 - t_1)/2$

$k_i = (t_{i+1} - t_{i-1})/2$ for $i = 2, 3, 4$; and

$k_5 = (t_5 - t_4)/2$

Replacing C_i by C'_i yields A'.

The difference between the two areas thus becomes

$$\bar{D} = A - A' = \sum_{i=1}^{5} k_i (C_i - C'_i)$$

To test the null hypothesis between the two areas one may use the statistic

$$t = \bar{D}/S_{\bar{D}}$$

where

$$S^2_{\bar{D}} = \sum_{i=1}^{5} k_i^2 (S_i^2 + S_i'^2), \quad S_i^2 \quad \text{and} \quad S_i'^2$$

being the estimates of the variances of the means for each group at time t_i. On the assumption that the mean concentrations follow normal distributions with different variances, Welch (1947) showed that the distribution of t may be approximated by Student's t-distribution with f degrees of freedom, where

$$f = \frac{\left[\sum_{i=1}^{5} k_i^2 (S_i^2 + S_i'^2)\right]^2}{\sum_{i=1}^{5} \frac{(k_i^2)^2 (S_i^2)^2}{n_i + 1} + \sum_{i=1}^{5} \frac{(k_i^2)^2 (S_i'^2)^2}{n_i' + 1}} - 2$$

n_i and n'_i being the number of mice in each group at time t_i in the control and cancerous group respectively.

Significance of the difference between control and leukaemic mice was assessed by the t-test and a P value of 0.05 or less was considered significant.

Results

As illustrated in Figure 1, peak sulphacetamide concentrations were reached within 10 min after drug administration in control mice. The drug was rapidly cleared from the blood as indicated by the relatively short half-life of the drug (Figure 2). In leukaemic mice, a very different pattern was found (Figure 1); peak drug concentration was reached 90 min after drug administration.

In the liver as well as in the spleen (Figure 1), the kinetics of sulphacetamide distribution was similar to that observed in the plasma for both control and leukaemic animals. The concentration of muscle sulphacetamide as a function of time was not markedly different from that of the above tissues except that peak drug concentration was not reached until 20 min after its administration (Figure 3). In leukaemic mice, there was a gradual increase in drug concentration as observed in other tissues. Sulphacetamide concentrations in the brain of control animals remained quite low and relatively constant throughout the observation periods (Figure 3). This is in contrast with leukaemic mice where, in the early periods of observation, lower brain concentrations of sulphacetamide were followed by constantly higher concentrations than in control animals; concentrations of the drug as a function of time are not unlike those observed in the other tissues of leukaemic mice. Except at the 20 min period, drug concentration in the fat tissue remained fairly constant in control animals (Figure 3). However, in leukaemic mice, there was progressive increase in the concentration of sulphacetamide in the tissue to reach a plateau 90 min after its administration.

Since up to 3.0 ml of ascitic fluid was recovered from leukaemic mice, it was important to measure the drug in that 'compartment'. Sulphacetamide concentrations in ascitic fluid were compared to those found in the plasma (Figure 4). Except for the 120 min period, sulphacetamide concentrations were found to be higher in the plasma than in the ascitic fluid.

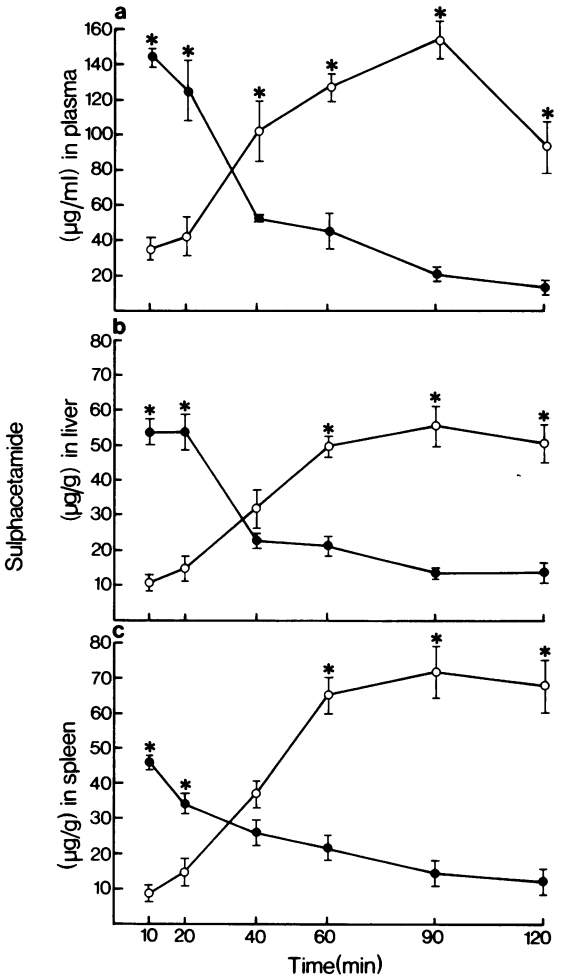

Figure 1 Sulphacetamide concentration in (a) plasma, (b) liver and (c) spleen at different times after oral administration of sulphacetamide sodium, 200 mg/kg in control (●) and leukaemic mice (L-1210) (○), 5 days after inoculation. Each point represents the mean results from 5 to 6 mice. Vertical lines show standard error. $*P<0.05$.

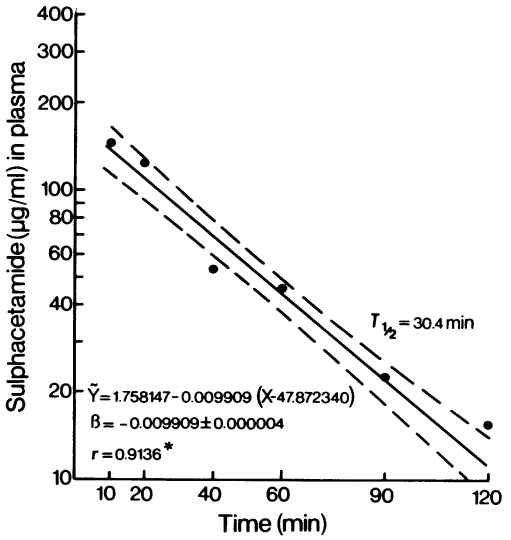

Figure 2 Relationship between the time of sulphacetamide sodium administration, 200 mg/kg and the log of sulphacetamide concentration in the plasma. The solid line is the estimated regression line: $\bar{y}=\bar{y}+\beta(x-\bar{x})$. The dotted lines are the 95% confidence limits. The correlation coefficient (r) is significant. Each point represents the mean of 5 to 6 mice.

Expressed in terms of percentage of the administered dose, sulphacetamide accumulation became important 40 min after its administration and reached a plateau at the 60 min period, with close to 5% of the administered dose recovered in the ascitic fluid.

As shown in Figure 5, sulphacetamide equilibrium between tissue and plasma was slow to be reached in control as well as in leukaemic mice. In all tissues of leukaemic animals, the tissue/plasma concentration ratios were found to be lower than those of control mice in the last periods of observation.

In order to assess the effect of cancer on drug absorption, sulphacetamide remaining in the stomach and the intestine was measured (Figure 6). Within the first 20 min following drug administration, more sulphacetamide was recovered from the stomach and the intestine of leukaemic mice.

The area under the curve of sulphacetamide plasma/concentration time curve (Figure 1) of control and leukaemic mice were compared. The AUC was found to be significantly larger in leukaemic mice than in controls: $\bar{D}=12778.04$; $S_{\bar{D}}=1372.16$; $t=9.31$; $f=35$; $P\leqslant 0.05$.

Discussion

Taking into consideration the higher metabolic rate and the faster circulation time in mice, the kinetics of sulphacetamide tissue distribution in this species is not unlike that observed in rats (Nadeau & Marchand, 1975). Concentrations of sulphacetamide as a function of time in the liver and muscle reflect those observed in the plasma. In the brain and fat tissue, the kinetics of sulphacetamide had a more specific pattern. However, contrary to what has been found in the rat, equilibrium between plasma sulphacetamide and other tissues was reached very slowly. Furthermore, a

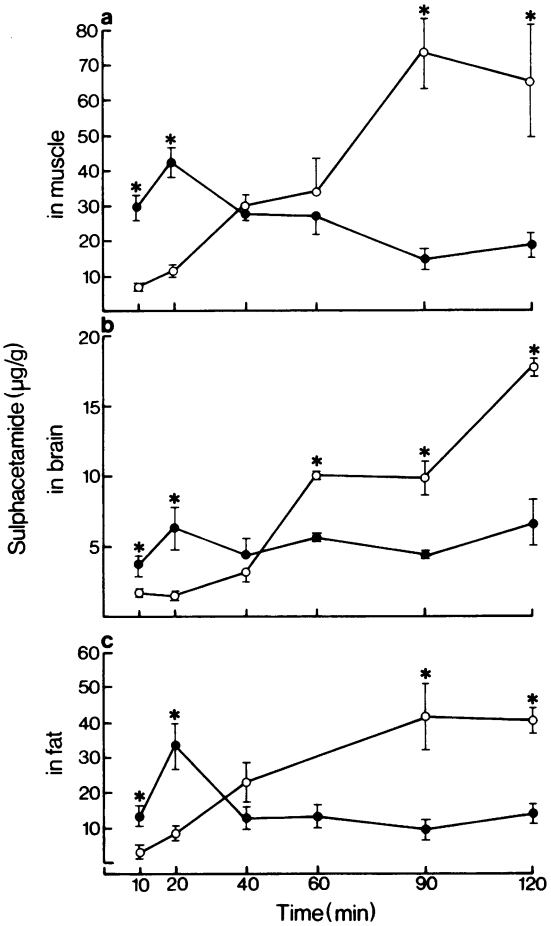

Figure 3 Sulphacetamide concentration in (a) muscle, (b) brain and (c) fat tissue at different times after oral administration of sulphacetamide sodium, 200 mg/kg, in control (●) and leukaemic mice (L-1210) (○), 5 days after inoculation. Each point represents the mean of results from 5 to 6 mice. Vertical lines show standard error. $*P<0.05$.

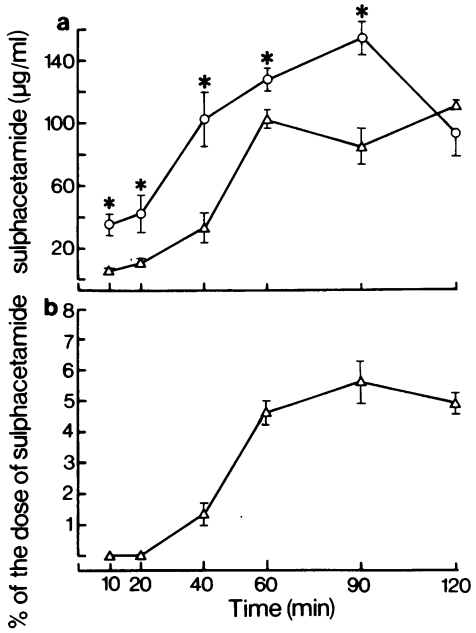

Figure 4 (a) Sulphacetamide concentration in the plasma (○) and ascitic fluid (△) and (b) accumulation in the ascitic fluid (△) at different times after oral administration of sulphacetamide sodium, 200 mg/kg in leukaemic mice (L-1210), 5 days after inoculation. Each point represents the mean results from 5 to 6 mice. Vertical lines show standard error. $*P<0.05$.

tissue/plasma ratio of one was reached in the muscle, liver and spleen of the mice whereas, in the rat, a ratio higher than 0.7 could never be found during the same period of observation.

The marked difference in plasma sulphacetamide kinetics in control and leukaemic mice may be partially explained by delay in gastrointestinal absorption in the leukaemic mice. The elimination phase of the drug was reached within the first 10 min in controls but not until the 90 min period in leukaemic animals. The hypothesis of delayed gastrointestinal absorption is reinforced by the greater amount of sulphacetamide recovered from the stomach and the intestine of leukaemic mice during the early periods following drug administration. There are many factors that could be responsible for the delay in the intestinal absorption of sulphacetamide in leukaemic animals (Wagner, 1961; Levine, 1970). One of these is the decrease in effective mucosal blood flow to the intestine (Ther & Winne, 1971) that could be associated with the emaciated state of these animals (Diamond, Doluisio & Crouthamel, 1970). However, there is also evidence that the large blood supply to the intestine makes it unlikely that diminution of blood flow may be responsible for decrease in the rate of drug absorption by passive diffusion (Varró, Blakó, Csernay, Jung & Szarvas, 1965; Lundgren, 1967). Another factor known to delay drug absorption in the intestinal tract is the slowing in gastric emptying (Bates & Gibaldi, 1970). This factor may play an important role in the delay of intestinal absorption of sulphacetamide since the stomach of leukaemic mice contained more than twice as much sulphacetamide as control animals during the first 20 min following drug administration. Biliary salts are known to increase the intestinal absorption of drugs in the dissociated form (Feldman & Gibaldi, 1969; Nightingale, Axelson &

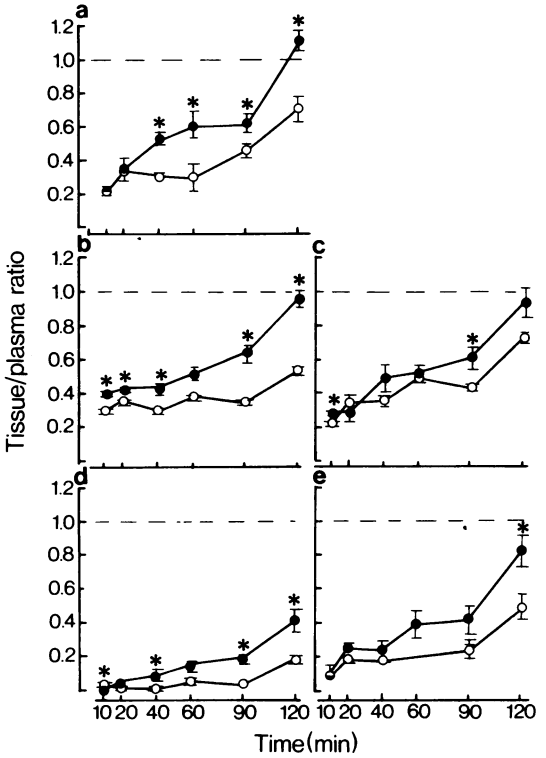

Figure 5 Tissue/plasma ratio of sulphacetamide concentration at different times after oral administration of sulphacetamide sodium, 200 mg/kg in control (●) and leukaemic mice (L-1210) (○), 5 days after inoculation: (a) muscle, (b) liver, (c) spleen, (d) brain and (e) fat tissue. Each point represents the mean from results from 5 to 6 mice. Vertical lines show standard error. $*P<0.05$.

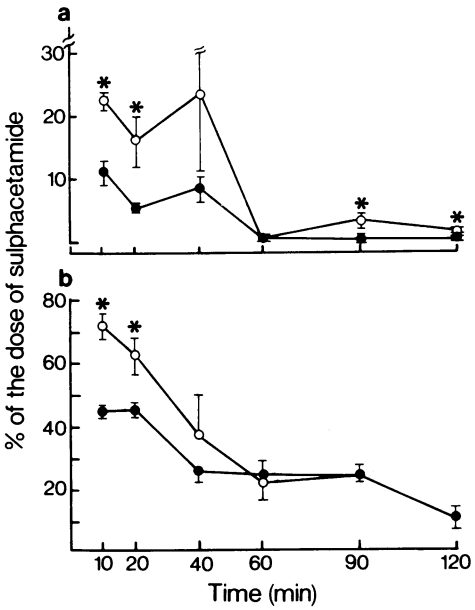

Figure 6 Percentage of the dose of sulphacetamide recovered from (a) the stomach and (b) the intestine at different times after oral administration of sulphacetamide sodium, 200 mg/kg in control (●) and leukaemic mice (L-1210) (○), 5 days after inoculation. Each point represents the mean results from 5 to 6 mice. Vertical lines show standard error. $*P<0.05$.

Gibaldi, 1971). Although we are unaware of published data dealing with the possible decrease of biliary excretion in leukaemic animals, hepatic dysfunction has been extensively reported in tumour-bearing animals (Greenstein, 1954; Homburger, 1957). It is also well known that factors that reduce the rate of cell renewal in the small intestine of animals, like starvation and protein depletion (Hooper & Blair, 1958; Takano, 1964; Deo & Ramalingaswami, 1965), cause a shortening of villi and absorptive surface is significantly reduced (Clark & Harland, 1963; Trier & Browning, 1966). Finally, any modification in the state of the epithelial membranes could modify the rate of drug absorption in the gut; given the extensive changes in the biochemistry of cancerous animals (Greenstein, 1954), it would not be surprising that the dynamics of the membranes of columnar epithelical cell be altered and drug absorption impaired.

The role of ascitic fluid in these changes of the kinetics of sulphacetamide observed in leukaemic mice is difficult to assess. Since there is little sulphacetamide recovered from the ascitic fluid within the first 20 min, it cannot explain the marked difference in tissue sulphacetamide concentrations observed during that period. However, ascitic fluid could act as an important reservoir for plasma sulphacetamide. This could explain the high drug concentrations observed during the last 60 min of the study.

Although sulphacetamide is apparently not metabolized to a great extent in the rat (Millburn, Smith & Williams, 1967; Marchand & Nadeau, 1973), there is no evidence that the mouse is similar to the rat in that respect. Inhibition of drug metabolizing enzymes in leukaemic mice could possibly explain the high plasma concentration of the drug in the late periods of observation (Kato *et al.*, 1963; Wilson, 1968, 1971; Rosso *et al.*, 1971). Impairment of renal function cannot be ruled out as a factor responsible for the high concentration of sulphacetamide in the plasma of leukaemic mice, one to two hours after drug

administration. Also, since these animals are likely to be in a catabolic state (Begg, 1958), a lowering in urine pH would also favour tubular reabsorption of sulphacetamide, pK 5.4 (Dettli & Spring, 1973). An increase in plasma protein, or affinity could possibly decrease sulphacetamide kidney filtration but opposite observations were made in rats: a decrease in plasma protein, as well as an increase in plasma free sulphacetamide were observed (Nadeau & Marchand, 1975).

The small concentrations of sulphacetamide in the brain of normal mice are consistent with previous findings in the rat (Goldsworthy, Aird & Becker, 1954; Nadeau & Marchand, 1975). However, contrary to these last observations, sulphacetamide concentration in the brain of control mice remained constant throughout the experimental periods whereas there was progressive accumulation of the drug in the brain of leukaemic mice. This could be interpreted as a change in the permeability of the blood brain barrier in leukaemic mice.

The lower tissue/plasma ratios observed in leukaemic mice are striking. It affects all tissues to varying degrees, and the differences are accentuated in the late periods of observation. It is difficult to explain these differences without invoking decrease in drug binding to tissue proteins in leukaemic mice (Gillette, 1973) or change in plasma and tissue pH favouring the ionization of sulphacetamide which would decrease diffusion of the drug from the plasma to tissues in leukaemic mice.

From the greater AUC in leukaemic mice, one would be tempted to conclude that drug bioavailability is increased in leukaemic mice. Although this conclusion cannot be ruled out, the greater AUC in leukaemic mice may simply be due to larger amounts of drug given to these mice because of their weights. This greater AUC could also be explained, in part, by the ascitic fluid that may be looked upon as a trap for sulphacetamide, preventing the drug from going to the cardiovascular compartment and preventing its excretion by the kidney; indeed, there seems to be a rapid equilibrium reached between drug in the plasma and the ascitic fluid.

From these and previous reports, one may conclude that the presence of cancer changes the distribution kinetics of sulphacetamide which could be due to delay in gastrointestinal absorption as well as trapping of the drug by ascitic fluid. These changes in the distribution kinetics coupled with decreased tissue/plasma ratios may have a bearing on the duration and intensity of drug action. It remains to be established that they are relevant to man.

This work was supported by the National Cancer Institute of Canada. The authors wish to thank Miss Claudette Lamoureux and Miss Claudette Doyon for their able technical assistance. We wish to acknowledge the invaluable contribution of Dr U. Maag, Département d'Informatique, Université de Montréal, who suggested the statistical analyses for the difference between areas.

References

ADAMSON, R.H. & FOUTS, J.R. (1961). The metabolism of drugs by hepatic tumors. *Cancer Res.*, **21**, 667-672.

BATES, T.R. & GIBALDI, M. (1970). Gastrointestinal absorption of drugs. In *Biopharmaceutics*, ed. J. Swarbrick, pp. 57-97. Lea & Febiger, Philadelphia.

BEGG, R.W. (1958). Tumor-host relations. *Adv. Cancer Res.*, **5**, 1-54.

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

CLARK, P.A. & HARLAND, W.A. (1963). Experimental malabsorption with jejunal atrophy induced by colchicine. *Br. J. exp. Path.*, **44**, 520-523.

DEO, M.G. & RAMALINGASWAMI, V. (1965). Reaction of the small intestine to induced protein malnutrition in rhesus monkeys. A study of cell population kinetics in the jejunum. *Gastroenterology*, **49**, 150-157.

DETTLI, L. & SPRING, P. (1973). Dosage regimens of sulphonamides based on pharmacokinetic parameters: principles and experimental results. Krueger Thiemer's theory on drug dosage: a simplified presentation. In *Biological Effects of Drugs in Relation to their Plasma Concentrations*, ed. D.S. Davies & B.N.C. Prichard, Baltimore, University Park Press.

DIAMOND, L., DOLUISIO, J.T. & CROUTHAMEL, W.G. (1970). Physiological factors affecting intestinal drug absorption. *Eur. J. Pharmac.*, **11**, 109-114.

FELDMAN, S. & GIBALDI, M. (1969). Bile salt-induced permeability changes in the isolated rat intestine. *Proc. Soc. exp. Biol.*, **132**, 1031-1033.

GILLETTE, J.R. (1973). The importance of tissue distribution in pharmacokinetics. *J. Pharmacokin. Biopharm.*, **1**, 497-520.

GOLDSTEIN, A. (1964). *Biostatistics, an Introductory Text.* pp. 129-147, New York, The MacMillan Co.

GOLDSWORTHY, P.D., AIRD, R.B. & BECKER, R.A. (1954). The blood-brain barrier. The effect of acidic dissociation constant on the permeation of certain sulfonamides into the brain. *J. cell Comp. Physiol.*, **44**, 519-526.

GREENSTEIN, J.P. (1954). *Biochemistry of Cancer.* pp. 507-588. Academic Press, Inc., New York.

HOMBURGER, F. (1957). *The biologic basis of cancer management.* pp. 148-150. Harper & Brothers, New York.

HOOPER, C.S. & BLAIR, M. (1958). The effect of starvation on epithelial renewal in the rat duodenum. *Expl. Cell Res.*, **14**, 175-181.

KATO, R., FRONTINO, G. & VASSANELLI, P. (1963).

Decreased activities of liver microsomal drug-metabolizing enzymes in the rats bearing Walker carcinosarcoma. *Experientia,* **19**, 31-32.

KATO, R., TAKANAKA, A. & OSHIMA, T. (1968). Drug metabolism in tumor-bearing rats. (II). *In vivo* metabolisms and effects of drugs in tumor-bearing rats. *Jap. J. Pharmac.,* **18**, 245-254.

LEVINE, R.R. (1970). Factors affecting gastrointestinal absorption of drugs. *Am. J. Dig. Dis.,* **15**, 171-188.

LUNDGREN, O. (1967). Studies on blood flow distribution and countercurrent exchange in the small intestine. *Acta Physiol. Scand.,* **303** (Suppl.), 3-42.

MARCHAND, C. & NADEAU, D. (1973). Effect of 2-Diethylaminoethyl-2, 2-Diphenylvalerate hydrochloride (SKF 525-A) on sulphacetamide distribution and excretion in rats. *Br. J. Pharmac.,* **47**, 69-76.

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

NADEAU, D. & MARCHAND, C. (1975). Change in the kinetics of sulphacetamide tissue distribution in Walker tumor-bearing rats. *Drug Met. & Dispos.,* **3**, 565–576.

NIGHTINGALE, C.H., AXELSON, J.E. & GIBALDI, M. (1971). Physiologic surface-active agents and drug absorption. VIII: effect of bile flow on sulfadiazine absorption in the rat. *J. Pharm. Sci.,* **60**, 145-147.

ROSSO, R., DONELLI, M.G., FRANCHI, G. & GARATTINI, S. (1971). Impairment of drug metabolism in tumor-bearing animals. *Eur. J. Cancer,* **7**, 565-577.

TAKANO, J. (1964). Intestinal changes in protein-deficient rats. *Exp. & Mol. Pathol.,* **3**, 224-231.

THER, L. & WINNE, D. (1971). Drug absorption. *A. Rev. Pharmacol.,* **11**, 57-70.

TRIER, J.S. & BROWNING, T.H. (1966). Morphologic response of the mucosa of human small intestine to X-ray exposure. *J. clin. Invest.,* **45**, 194-205.

VARRÓ, V., BLAKÓ, G., CSERNAY, L., JUNG, I. & SZARVAS, F. (1965). Effect of decreased local circulation of the absorptive capacity of a small-intestine loop in the dog. *Am. J. Dig. Dis.,* **10**, 170-177.

WAGNER, J.G. (1961). Biopharmaceutics: absorption aspects. *J. Pharm. Sci.,* **50**, 359-387.

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

WELCH, B.L. (1947). The generalization of 'Student's' problem when several different population variances are involved. *Biometrika,* **34**, 28-35.

WILSON, J.T. (1968). An investigation of the decrease in the metabolism of hexobarbital, aminopyrine and *p*-nitrobenzoic acid by liver from rats bearing a pituitary mammotropic tumor. *J. Pharmac. exp. Ther.,* **160**, 179-188.

WILSON, J.T. (1971). Altered rat hepatic drug metabolism after implantation of a pituitary mammotropic tumor (M_tT), Walker carcinosarcoma or adenocarcinoma and after removal of the M_tT. *Endocrinology,* **88**, 185-194.

(Received August 5, 1975.
Revised November 7, 1975.)