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Influence of the Route of Administration on the Area Under the Plasma Concentration-Time Curve

P. A. HARRIS* and S. RIEGELMAN

Abstract □ The so-called law of corresponding areas has been used to assess the percent of absorption by comparison of the relative areas under the plasma concentration-time curves after oral and intravenous administration. These analyses are based on the presumption that the resultant areas are independent of the route of administration. After oral administration, every absorbed molecule must pass through the gut wall and when absorbed *via* the hepatic portal system must traverse through the liver, before reaching the central compartment, from which samples are obtained for analysis. However, after the usual i.v. administration, less than 30% of the molecules traverse through the liver in the first circulatory pass. Therefore, if a significant degree of metabolism takes place in the gut or in the liver, the resultant areas will not be identical. This has been verified in dogs by comparison of areas after infusion of acetylsalicylic acid by the vena cava, by the hepatic portal vein, and after oral administration. The data obtained with acetylsalicylic acid indicate metabolism occurs in both the gut wall and in the liver, thereby causing a large reduction in the areas obtained after oral dosing or hepatic portal vein infusion when compared to the area obtained after infusion by the vena cava.

Keyphrases □ Plasma concentration-time curve area—administration route □ Acetylsalicylic acid—i.v., oral administration □ Areas, concentration-time curves—i.v., oral administration □ GLC—analysis □ Fluorometry—analysis

Dost (1) and Gladtke (2, 3) have shown that the area under the plasma concentration-time (PCT) curve is proportional to the dose of drug administered. They utilized *p*-aminohippuric acid and some sulfonamides in their experiments and administered these compounds intravenously and orally. The proportionality between dose and the area under the PCT curve has been referred to as "the law of corresponding areas" and is based on the presumption that distribution, metabolism, and excretion may be expressed in terms of first-order kinetics within the dose ranges studied.

The model in Fig. 1 for the disposition of a drug in the body depicts the manner in which the rate of metabolism and elimination may be proportional to the plasma concentration. This system is an open two-compartmental model in which *Cp*, the plasma concentration, represents the concentration in Compartment 1 and *Ct* represents the concentration in Compartment 2. Metabolite(s) and excreted unchanged drug are represented by *ME* and *kel* represents the first-order rate constant of metabolism and excretion. This model has been shown to describe the disposition in animals of many exogenous compounds. Among such compounds are *p*-aminohippuric acid (4), creatinine (5), aldosterone (6), and acetylsalicylic acid (7). The proportionality between dose and the area under the PCT curve in the model shown in Fig. 1 is based on the following differential equation,

$$\frac{dM}{dt} + \frac{dE}{dt} = \frac{dME}{dt} = kel\ VpCp \qquad \text{(Eq. 1)}$$

where $kel = ke + km$, the first-order rate constants of excretion and metabolism, respectively. The total amount of the metabolized and excreted compound is represented by *ME*, the plasma concentration by *Cp*, and the volume of Compartment 1 by *Vp*. Since it is

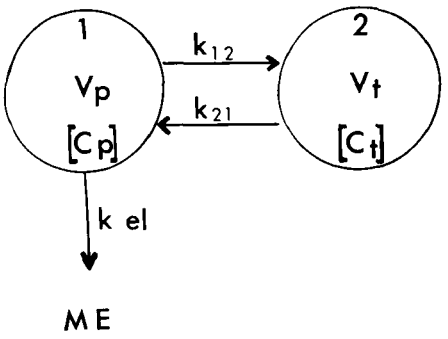

Figure 1—*Schematic diagram of a two-compartmental model for drug disposition in the body. Compartment 1 is called the central compartment with a volume,* V_p, *whose concentration is represented by the plasma concentration,* C_p. *Compartment 2 is called the peripheral compartment with a volume,* V_t, *and a concentration* C_t *both expressed relative to* V_p *and* C_p. *All metabolites and excreted unchanged drug are represented by* ME. *The first-order rate constant,* kel, *represents all elimination processes and* k_{12} *and* k_{21} *represent the first-order transfer constants of distribution between Compartments 1 and 2.*

presumed that all processes of elimination are represented by Eq. 1, integration between time zero and infinity yields

$$ME^{\infty} = \text{dose} = kel\ Vp \int_0^{\infty} Cpdt \qquad \text{(Eq. 2)}$$

where $\int_0^{\infty} Cpdt$ represents the area under the PCT curve.

An important point represented by the model and Eqs. 1 and 2 is that elimination (metabolism and excretion) occurs from Compartment 1 of which the plasma is a part. The dose and area may still be proportional if elimination does not exclusively occur from Compartment 1 but they will not be related by the product of *kel* and *Vp* which is the body clearance of the substance administered.

Gladtke and Mohos have shown the proportionality between the dose of *p*-aminohippuric acid and the area under the PCT curve is independent of the rate by which the compound is introduced into the body (2). Gladtke later reported (3) on two sulfonamides which were administered intravenously and orally. The resulting areas under the PCT curves were shown to be proportional to the dose even when given by different routes. All of the compounds used in the two aforementioned reports are negligibly or slowly metabolized and therefore may not be appropriate to test the applicability of the law of corresponding areas to different routes of administration.

Previous investigations in this laboratory (8) have shown that the area under the PCT curve from an intravenous dose of acetylsalicylic acid (ASA) was greater than the area under the PCT curve from an equal oral dose of ASA. That ASA was completely absorbed from the oral dose, was demonstrated by the equal area under the PCT curves obtained from the metabolite, salicylic acid (SA), after the two modes of administration.

The purpose of this investigation is to define the effects of the route of administration upon the area under the PCT curve of ASA, a compound which is metabolized at an appreciable rate. This investigation will also attempt to show how these effects can be utilized to further the knowledge of the metabolism of drugs using ASA as a test compound.

EXPERIMENTAL

Animal Preparation—Male mongrel dogs weighing between 18 and 23 kg. were used as experimental subjects. The dogs were anesthetized with pentobarbital (25–30 mg./kg. i.v.) and given additional doses as needed during surgery.

The abdomen was opened through a midline incision extending from the xyphoid process to the navel. One end of a catheter was introduced into a convenient branch of the splenic vein near its origin. The catheter was guided upward into the hepatic portal vein so that its tip projected several centimeters into this vein. The other end of the catheter was exteriorized through a stab incision in the left flank posterior to the 13th rib.

Through appropriate incisions the right femoral artery and the left femoral vein were catheterized. The catheters were extended into the lower abdominal aorta and inferior vena cava, respectively. They were then exteriorized by running them under the skin through the loose subcutaneous tissue cranially and to the left side and out the same exit as the hepatic portal vein catheter.

After the abdominal incision was closed the three catheters were brought out near the spinal column by reintroducing them into their original exit through the skin and continuing further dorsally and subcutaneously as before. The catheters were then filled with heparin solution, 300 units/ml., and clamped with light plastic screw clamps. An antibiotic ointment containing neomycin, 5 mg./g., polymyxin B sulfate, 5000 units/g., and zinc bacitracin 400 units/g. was used around the catheter exit in the skin to prevent infection. The catheters and clamps were wrapped in gauze and tape and secured under several layers of 4-in. stockinette wrapped around the dog's midsection.

Catheters—All the catheters consisted of silicone gum rubber,[1] o.d. 0.27 cm. (0.105 in.), i.d. 0.13 cm. (0.053 in.). This type of catheter material has little tendency to cause clot formation. The catheters were flushed every 3 days with fresh heparin solution (300 units./ml.) and most preparations were still potent several weeks after implantation.

Dose Formulation—Acetylsalicylic acid (USP)[2] was used as the test drug and was given in solution. Solutions for oral administration were prepared by wetting 250 mg. of ASA with 2 ml. of ethyl alcohol and then adding 150 ml. of water and shaking 2 or 3 min. to effect solution. The ASA solutions for intravenous administration were prepared by wetting 250 or 500 mg. of ASA with 2 ml. of ethyl alcohol then adding sterile distilled water containing an equal molar quantity of *N*-methyl glucamine[3] to make between 20 and 50 ml. All solutions were prepared within 1 hr. of use and were kept in an ice bath until used.

Animal Experiments—The dogs were fasted for 18 hr. prior to the experiments but had free access to water. Experiments were performed at least 7 days after surgery and were carried out in unanesthetized dogs trained to lie still during the experiments. Doses of ASA were administered by constant infusion *via* the vena cava (VC) catheter and hepatic portal vein (HP) catheter over a period of 13 to 24 min. Oral doses of ASA were given within 1 min. through a stomach tube introduced just prior to the experiment Three dogs were given 250-mg. doses by the VC, HP, and oral routes in random order. A fourth dog was given 250 and 500 mg. by the VC and HP routes. Serial blood samples (10–15 of 3 ml. each) were taken from the aortic catheter during and after administration of ASA for analysis of ASA and SA concentrations. With Dogs 1 and 2 sufficient time was allowed between the 250-mg. doses given by different routes for the ASA to completely disappear and for the SA to reach a maximum level. Dogs 3 and 4 received each different dose on successive days so that not only had the ASA disappeared from the previous dose but the SA as well.

Handling of Blood Samples and Analysis of Plasma—Blood samples obtained from the dogs were immediately added to test tubes containing 50 μl. of potassium fluoride solution, 50% w/v.

[1] Vivosil, Bard-Parker Co., Inc., Rutherford, N. J. or Silastic, Dow Corning Corp., Midland, Mich.
[2] Merck and Co., Inc., Rahway, N. J.
[3] Kindly furnished by Dr. W. Moore of Sterling-Winthrop Research Institute.

Table I—Areas Under Plasma Concentration-Time Curves After Different Routes of Administration of ASA

Subject	Route	Time Taken for Delivery of Dose, min.	Dose, mg.	Area[a] Under PCT Curve of ASA, mcg. min./ml./mg.	Relative Area[a]
Dog 1	VC	13	250	2.08	1.00
	HP	13	250	1.23	0.59
	Oral	<1	250	0.95	0.46
Dog 2	VC	20	250	1.66	1.00
	HP	20	250	1.30	0.78
	Oral	<1	250	0.86	0.52
Dog 3	VC	24	250	2.47	1.00
	HP	24	250	1.34	0.54
	Oral	<1	250	0.91	0.37
Dog 4	VC	22.5	250	1.11	1.00
	HP	22.5	250	0.71	0.64
	HP	22.5	500	0.75	0.68
	VC	22.5	500	1.07	0.96

[a] See *Results* section for calculations.

Centrifugation of the blood samples was carried out within 20 min. in prechilled tube holders. The plasma was separated immediately, placed in vials, and stored in dry ice. The plasma was analyzed for ASA content by GLC and for SA content by spectrophotofluorometry. The details of the handling of blood samples and analysis of ASA and SA in plasma have been previously described (9).

RESULTS

The areas of the PCT curves obtained after administering ASA by the VC, HP, and oral routes are listed in Table I. The areas were calculated by resolving the area under the curve into small increments and applying the formula for trapezoidal areas to each increment and summing the incremental areas. The PCT areas in Table I are expressed as mcg. min./ml./mg. of administered dose. The relative area in Table I expresses the fractional PCT area of any given dose or route of administration compared to the PCT area of the 250-mg. VC dose.

The areas obtained from HP administration of ASA are 54 to 78% of those obtained by VC administration. Oral dosages yielded even lower percentage areas of 37 to 52%.

The graphical illustration of the ASA PCT curves obtained after ASA administration in Dog 3 is shown in Fig. 2. The low, prolonged, and relatively constant plasma level of ASA obtained after oral administration appeared similar to that shown for Dog 3 in all dogs.

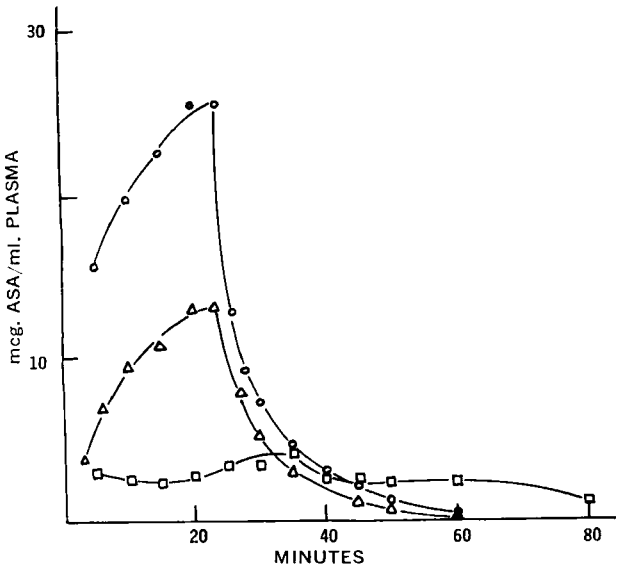

Figure 2—*Plasma concentration-time curves of ASA obtained after administration of 250 mg. ASA by various routes in Dog 3. Key:* O, *VC route;* △, *HP route;* □, *oral route.*

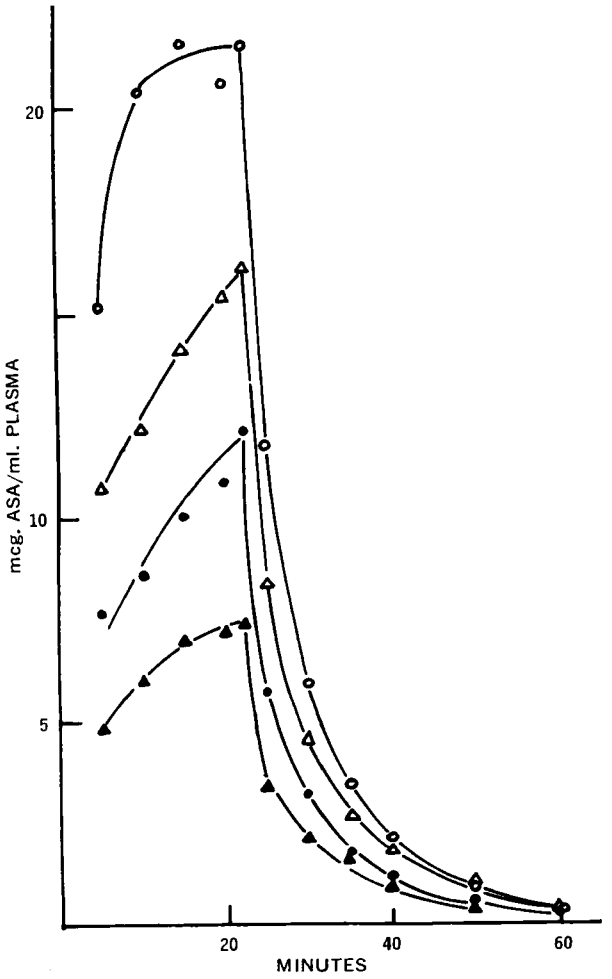

Figure 3—*Plasma concentration-time curves of ASA obtained after administration of 250 or 500 mg. ASA by VC and HP routes in Dog 4. Key:* ●, *VC, 250 mg.;* ▲, *HP, 250 mg.;* O, *VC, 500 mg.;* △, *HP, 500 mg.*

Dog 4 was given two different amounts of ASA by the HP and VC routes. The curves obtained from these experiments are shown in Fig. 3. The area from the 500-mg. VC dose was twice that obtained from the 250-mg. VC dose. Likewise, the area from the 500-mg. HP dose was twice that from the 250-mg. HP dose.

The plasma level of SA in Dog 3 rose faster after HP administration of ASA than after VC administration, while after oral administration the rate of rise was by far the slowest (Fig. 4). The total areas of the SA PCT curves from all three routes were identical within experimental error. Similar results for SA were obtained in experiments with Dog 4.

DISCUSSION

The magnitude of the area under the PCT curve of any given compound is dependent upon the extent of distribution of the compound in the body, its rate of elimination from the body, and the quantity of the compound delivered to the particular vascular site being sampled. It can be assumed that the parameters of distribution and elimination remain constant after administering the same quantity of a compound to the body by two different routes. If the areas obtained after the two doses are unequal, then it must be concluded that more of the intact compound reaches the vascular site being sampled from one route than the other. The most likely manner by which this may occur is when one route of administration results in the compound passing through an organ which will metabolize or excrete part of the compound before it reaches the sampling site.

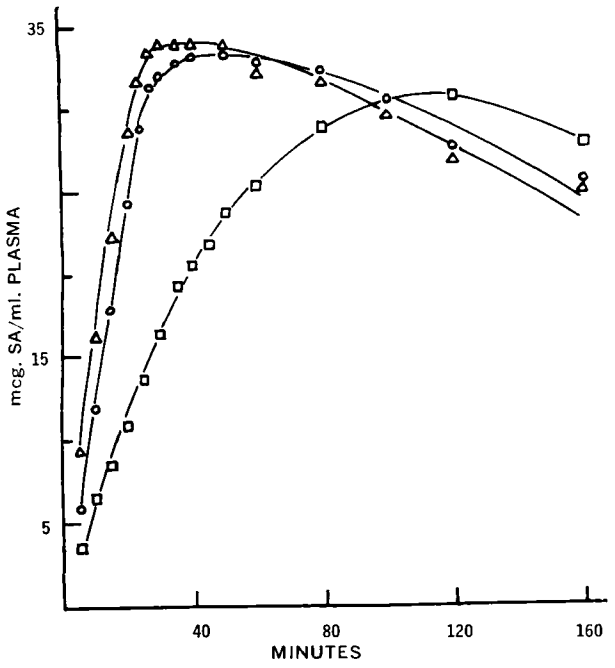

Figure 4—*Plasma concentration-time curves of SA obtained after administration of 250 mg. ASA by various routes in Dog 3. Key: O, VC route; △, HP route; □, oral route.*

For example, were one to infuse a given dose of *p*-aminohippuric acid (PAH) intravenously and at a later time into the renal artery, one would observe a large difference in the area under the PCT curves obtained from analyzing PAH in mixed venous or peripheral arterial blood. The difference results from the fact that all of the molecules would traverse the kidney from the renal artery infusion and 80 to 90% would be extracted and excreted into the urine on a single pass through that kidney. Therefore, a large proportion of the PAH infused into the renal artery would never reach the mixed venous or arterial sampling site. Furthermore, the percent reduction in the area from the renal artery infusion compared to the intravenous infusion would correspond to the percent of PAH extracted on a single pass through that kidney, providing the average blood flow to the kidneys and the parameters of PAH extraction and distribution did not change from one infusion to the other. A similar result should be expected for a compound which is cleared to a significant extent in any organ.

The results obtained with ASA in this investigation are given in Table I. They suggest that a considerable percentage of the ASA which enters the liver *via* the hepatic portal vein is hydrolyzed to SA before entering the vena cava. This is indicated by the lower areas under the PCT curves after HP administration as compared to VC administration. The values for the area after the HP dose were 59, 78, 54, and 64% of the VC dose. This corresponds to 41, 22, 46, and 36% metabolism on the first pass through the liver.[4]

Leonards (10) has reported a difference in ASA concentration between the hepatic portal vein and the hepatic vein by drawing serial blood samples from both veins simultaneously *via* implanted catheters in dogs. The blood samples were obtained at 10-min. intervals from 10 to 60 min. after an oral dose of ASA in solution. One can calculate the percentage metabolized on a pass through the liver from the portal vein and hepatic vein concentrations reported by Leonards. If this is done, the result is a gradually decreasing apparent metabolism from 71% at 20 min. to 48% at 60 min.

There are many pitfalls in measuring plasma-concentration differences (A-V differences) across an organ which have been discussed in detail by Zierler (11). He indicates that one prerequisite for A-V difference measurements is that the compound being measured must be in steady state within the body tissues and fluids as may be indicated by a prolonged constant plasma concentration. Generally, the oral route of administration of ASA as used by Leonards is not conducive to the attainment of the steady-state condition since the rate of delivery from the gut to circulation may not be constant. A constant rate of delivery is necessary for the steady-state condition in order for the tissues to come into distribution equilibrium with the plasma. The A-V differences reported by Leonards probably do not represent metabolism alone, but in addition include some distribution, particularly in the earlier time intervals. However, data reported here (Fig. 2) indicate absorption of ASA from solution in the dog may be relatively constant. Difference values at the later time points in Leonards' study approach the values given by the area method in this report. This is consistent with the possibility that the steady-state condition was being approached where A-V differences would have been more meaningful. Also consistent with the steady-state requirement, it has been found (12) that the A-V difference of ASA across the liver in the steady-state condition, attained by prolonged constant intravenous infusion, corresponds closely with the difference in the areas under the PCT curves following HP and VC administration.

The results obtained in Dog 4 (Fig. 3) indicate that the area under the PCT curve is proportional to the dose administered at least within the range used here. The fact that there was a doubling of the area of the PCT curve after a 500-mg. HP dose compared to a 250-mg. HP dose indicates that the hydrolytic process in the liver was not saturated or otherwise kinetically different in the two doses. As mentioned previously, the area under the PCT curve from an oral dose of ASA was less than that of an HP dose. However, if metabolism occurred in the gut lumen or wall, then the area obtained from the oral dose should be lower than that from the HP dose due to this additional organ metabolism. One might expect the area from an oral dose to be the same as an HP dose, when absorption is complete, since all the molecules must pass into the hepatic portal circulation and traverse the liver.[5] Figure 4 includes the data on the SA levels found after administration of ASA by the various routes. The areas under the complete PCT curves were identical within experimental error indicating that absorption was complete from the oral dose. Typical data on the ASA levels after oral administration are shown in Fig. 2 and the area analyses in Table I. In the three dogs where the levels of ASA after oral doses were measured the areas were 46, 52, and 37% of the VC areas. Relative to the HP areas these studies indicate 22, 30, and 32% was metabolized[6] in the gut wall or lumen. Metabolism in the gut lumen is not likely since a previous communication from this laboratory (8) reported the lack of significant ASA hydrolytic activity in gastric and duodenal fluids from humans.

Considering the results of this investigation, one must use caution in applying the law of corresponding areas as developed by Dost and Gladtke in the assessment of drug absorption, especially from the gastrointestinal tract. Appreciable clearance or metabolism occurring in the gastrointestinal tract or hepatic tissues will result in a lower area under the PCT curve after an oral dose when compared to an intravenous dose even though absorption may be complete. If the areas obtained from different dosage forms given orally are compared, one must still consider the possible consequences of metabolism in the gut or liver since these processes are sometimes saturable. This has been shown for salicylamide in humans for both hepatic and gut glucuronidation (13).

The only present method known to distinguish between these events, organ clearance *versus* incomplete absorption, from blood-level data is to compare the areas under the PCT curves for both the intact compound and one of its metabolites. Utilizing as a standard, the area under the PCT curves of the metabolite and intact compound obtained after intravenous administration of the latter, one can distinguish between four possible combinations of events: complete absorption with and without appreciable organ clearance and incomplete absorption with and without organ clearance. On this basis the present investigation results in evidence for complete

[4] Calculated on the following basis:

$$\text{percent metabolized} = 100 \times \frac{\text{VC area} - \text{HP area}}{\text{VC area}}$$

[5] Two assumptions are made in the above statement: absorption *via* the intestinal lymph channels does not occur and absorption is complete before the rectal area is reached where part of the hemorroidal veins drain into the vena cava instead of the portal circulation.

[6] Calculated on the following basis:

$$\text{percent metabolized} = 100 \times \frac{\text{HP area} - \text{oral area}}{\text{HP area}}$$

absorption from the oral route with gut and hepatic clearance (metabolism) of ASA. If, however, the oral to VC areas under the metabolite curves differed, but their ratio was greater than the ratio of the intact compound then this would indicate both incomplete absorption and organ clearance.

SUMMARY

The influence the route of administration may have on areas under plasma concentration-time curves is discussed.

It is shown that in dogs, acetylsalicylic acid is metabolized in the liver and in the gastrointestinal tract to a considerable extent.

The effect of hepatic and gastrointestinal metabolism of ASA when ASA is administered orally or *via* the hepatic portal vein is to reduce the area under the plasma concentration-time curves compared to the area of an equal intravenous dose.

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Nucleosides in Human Urine I: Isolation and Identification of N_2-Dimethylguanosine, 1-Methylinosine, and N_2-Methylguanosine from Normal Human Urine

GIRISH B. CHHEDA, ARNOLD MITTELMAN, and JAMES T. GRACE, JR.

Abstract □ Using partition column chromatography, three methylated nucleosides have been isolated from normal human urine. The lyophilized urine was fractionated on a diatomaceous earth column using a formic acid gradient in an ethyl acetate–2-ethoxyethanol solvent system. Purification of the major fractions, using repeated paper chromatography, led to the isolation of methylated nucleosides, N_2-dimethylguanosine, 1-methylinosine, and N_2-methylguanosine. The identity of these nucleosides was established by comparison of their paper chromatographic and electrophoretic mobilities and UV spectra with those of authentic samples. Acid hydrolysis and the orcinol color reaction were in agreement with their identity as nucleosides.

Keyphrases □ Nucleosides—human urine □ N_2-dimethyl- and methylguanosine, urine—isolation, identification □ 1-Methylinosine, urine—isolation, identification □ Column chromatography—separation □ Paper chromatography—separation, identification □ UV spectrophotometry—analysis

In the last fifteen years, a number of workers (1–10) have contributed to the growing list of urinary purines and pyrimidines that most probably are metabolites of nucleic acids. In particular, Weissmann *et al.* (3–5) have characterized several methylated purines in normal human urine. The elevated levels of the methylated purines excreted by leukemic subjects was observed by Adams *et al.* (6) and by Park *et al.* (11).

It is well established that the minor nucleosides occur mostly in transfer ribonucleic acid (tRNA) (12, 13) and to a small extent in ribosomal RNA (13). The minor bases isolated from urine appear to be the metabolic end products of tRNA (14). Thus, in order to study the metabolism of tRNA in normal and diseased human subjects, it was of importance to determine if any of the nucleosides were excreted in normal human urine in addition to the methylated bases. Reports in the literature indicate that pseudouridine is the most commonly found minor nucleoside of tRNA in urine (7). Fink *et al.* (10) have characterized N_2-dimethylguanosine from one urine sample and the base N_2-dimethylguanine from several normal and leukemic human urine samples.

The methodology developed by Weissmann *et al.* (3) for the isolation of methylated purines cannot be applied to the isolation of nucleosides, since it involves