Table IV—Statistical Analysis of the Survival Studies on an SPSS Multiple Regression Subprogram

Drug	Dose Range	R^2 [a]	a [b]	b [b]	c [b]	$B(b)$ [c]	$B(c)$ [c]	$F(b)$ [d]	$F(c)$ [d]
Ethyl ether	25–100	0.183	8.982	0.0137	−0.995	0.377	−0.365	8.3	7.8
	125–200	0.387	15.714	−0.0463	NS [e]	−0.623	NS	22.62	NS
Propyl ether	25–100	0.103	9.677	0.0155	NS	0.3219	NS	4.950	NS
	125–200	0.349	15.262	−0.0433	NS	−0.591	NS	19.365	NS
Butyl ether	25–100	0.268	8.556	0.0333	NS	0.518	NS	5.854	NS
	25–150	0.237	9.683	0.0138	−2.189	0.235	−0.433	4.993	16.96
Hexyl ether	25–200	0.0221	10.21	−0.0028	NS	−0.149	NS	2.391	NS

[a] R^2 (coefficient of determination) indicates the proportion of variation in life span explained by dose only or by both dose and treatment. [b] The form of multiple regression equation: $Y = a + b(\text{dose}) + c(\text{treatment})$ Y = life span, a = intercept of y axis, b = regression coefficient for dose, c = regression coefficient for treatment (dummy variable). [c] $B(b)$ indicates the number of standard derivation units of change in life span that could be predicted when dose changes by one standard unit. $B(c)$ indicates the number of standard derivation units of change in life span that could be predicted when treatment changes by one standard unit. [d] $F(b)$ presents the F value of dose. $F(c)$ presents the F value of treatment. Except $F(b)$ of hexyl ether, all other F values are greater than the critical values at the 0.05 level of significance. [e] NS = no significance.

on toxicity or survival times in leukemic mice would be expected to result from phenobarbital pretreatment (3, 4). It is not known if the hexyl ether of *p-N,N-*bis(2-chloroethyl)aminophenol undergoes (ω-1)-hydroxylation, but if it were to occur, these results suggest a metabolite similar in toxicity and antitumor activity to compound V.

A regression analysis was performed on survival data from the control and pretreated groups (Table IV). The analysis, taken over the entire range of doses, failed to show significant trends for any of the compounds tested. Negative trends in the ascending points for data sets from compounds II and IV indicate a decrease in the antitumor effectiveness for the pretreated mice. Crossover trends at the lowest and two highest doses for compound III precluded significance. The medium level doses were not tested. No trend could be established for compound V.

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Antipyrine and Acetaminophen Kinetics in the Rat: Comparison of Data Based on Blood Samples from the Cut Tail and a Cannulated Femoral Artery

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Abstract ☐ Antipyrine and acetaminophen kinetics were determined from concentration data obtained by simultaneous blood sampling from the cut end of the tail and a cannulated femoral artery in the rat. Significant differences in concentrations and kinetics for both drugs were found by comparison of the two sampling sites. The hypothesis that the differences were due to a low tail blood flow was tested. The tail blood flow was measured with a microsphere technique, and tail antipyrine concentrations were calculated from the relationship between arterial antipyrine concentration, tail flow, and time for comparison with the observed antipyrine concentrations. Mean blood flow of the rat tail was 0.02 ml/min/ml tail tissue at 22°, which was 8.8 and 0.9% of the liver and kidney flow, respectively. Tail flow increased more than twofold by elevation of the tail temperature to 37°. The calculated tail antipyrine concentration *versus* time curve showed a very close correspondence to the observed antipyrine tail concentration *versus* time curves. The results show that tail flow is a major determinant of antipyrine tail concentration in the rat. Kinetic data based on blood samples from the cut end of the tail, therefore, should be interpreted with caution.

Keyphrases ☐ Antipyrine—pharmacokinetics in the rat, comparison of data based on blood samples from the cut tail and a cannulated femoral artery ☐ Acetaminophen—pharmacokinetics in the rat, comparison of data based on blood samples from the cut tail and a cannulated femoral artery ☐ Pharmacokinetics—acetaminophen and antipyrine in the rat, comparison of data based on blood samples from the cut tail and a cannulated femoral artery ☐ Microsphere technique—pharmacokinetics in the rat, comparison of data based on blood samples from the cut tail and a cannulated femoral artery

The development in recent years of a multitude of sensitive drug assays in microsamples of blood and a variety of techniques for repeated blood sampling have made it possible to perform pharmacokinetic studies in individual animals as small as mice, rats, and guinea pigs. Whereas published methods for drug assays are routinely validated

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with respect to their sensitivity, specificity, and precision, methods for blood sampling in small animals mostly have been evaluated as to their feasibility in practical laboratory work (1, 2). The widespread use of small animals in pharmacokinetic studies of old and new drugs, however, has created a need for precise information also of the reproducibility and validity of the methods used for blood sampling. Recently, the reproducibility and validity of pharmacokinetic data obtained with a surgical procedure for cannulation of an artery and vein in the rat was investigated (3).

The present study was undertaken to investigate a frequently employed nonsurgical method: sampling of blood from the cut end of the rat tail. Assuming a low flow to the rat tail (4), both theoretical considerations (5–8) and previous experience (9) raised doubts about the validity of tail concentration data. Significant differences in antipyrine and acetaminophen concentrations in blood sampled simultaneously from the cut end of the tail and a cannulated femoral artery are presented. A low blood flow of the rat tail is demonstrated, to which the differences in tail and arterial antipyrine concentrations are attributable.

EXPERIMENTAL

Animals and Operation—Male Wistar rats[1] (150–400 g) were used. All surgery was performed in fluanisone/fentanyl[2] anesthesia (6.6/0.13 mg/kg sc) in the evening, and the experiments were carried out the next day. Arteries and veins were cannulated with polyethylene tubing[3] previously filled with 0.2 ml of heparinized (100 IU/ml) 0.9% NaCl, with the indwelling part of the tubing having been stretched to reduce its diameter and lubricated with silicone oil to facilitate insertion. For pharmacokinetic experiments a femoral artery and a femoral vein were cannulated; for blood flow measurements, the right carotid and a femoral artery were cannulated. The tubing was secured and transferred dorsally through a subcutaneous tunnel and made accessible through a skin perforation. The rats were placed in restraining cages[4] and allowed free access to food and water. Sampling of blood from the tail was performed after removal of its distal part (0.5 cm) with a sharp scalpel by a previous method (10), but without prewarming of the rat and occlusion of the lateral tail veins. The tail was allowed to bleed freely, usually a few seconds after blood sampling, and no hemostat was applied. Repeated bleeding was obtained by removal of the blood clot from the end of the tail and the samples collected in heparinized capillary tubes.

Pharmacokinetic Experiments—Experiments were carried out in unanesthetized rats. The test drugs, dissolved in 0.6–0.9 ml of physiological saline, were infused *via* the catheter in the femoral vein. The infusion period (30 sec) was immediately followed by flushing of the catheter with 0.3 ml of saline. An equal dose of [^{14}C]antipyrine[5] and [^{3}H]acetaminophen[6] was given (15 mg/kg, 1–2 μCi/animal). Blood samples (0.1 ml) were drawn from the cannula in the femoral artery and the cut end of the tail. After dosing with antipyrine, blood samples were drawn at 2, 4, 8, 10, 15, 20, 35, 50, 75, 100, 135, 160, and 200 min. After dosing with acetaminophen, blood samples were drawn at 2, 4, 6, 8, 12, 20, 30, 40, 50, and 60 min.

The dead space of the catheter was discarded before each sampling and replaced by saline afterwards. Concentrations of [^{14}C]antipyrine and [^{3}H]acetaminophen in whole blood were analyzed essentially by the methods described previously (11, 12). These assays have been shown to measure specifically only unchanged antipyrine or acetaminophen, with 95 and 90% recovery of the drugs from blood, respectively (11, 12).

Concentrations of antipyrine and acetaminophen were plotted *versus* time on semilogarithmic graphs. Arterial and tail blood concentrations exhibited biexponential and monoexponential decay curves, respectively. The arterial blood concentrations were analyzed according to a two-compartment open model with first-order elimination kinetics. The best fitting line was drawn by means of a formula of linear regression of the natural logarithm of concentration *versus* time based on the method of least squares.

Total clearance was calculated by:

$$\text{dose}/(A/\alpha + B/\beta) \qquad \text{(Eq. 1)}$$

where A and B are γ-intercepts of the extrapolated lines of the α- and β-phases, respectively. The central volume of distribution (V_c) was obtained by dividing the dose by ($A + B$), and V_β was calculated by dividing total clearance by β.

Data obtained from tail blood were analyzed as follows: The area under the observed concentration curves were calculated by the trapezoidal rule and the total area under the curve by the equation:

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_t}{\beta} \qquad \text{(Eq. 2)}$$

assuming that C_t represents a point on the concentration curve where a linear terminal line can be drawn (pseudodistribution equilibrium). Total clearance was calculated by dose/$AUC_{0-\infty}$ and V_β by dividing total clearance by β.

Determination of Tail Flow—Blood flow through the rat tail was measured by means of the radioactive microsphere technique for determinations of regional blood flow (13).

Calculation of the Blood Flow—The blood flow through each piece of the tail was calculated by (14):

$$Q = \frac{F_r \, i_{x/g}}{i_\gamma} \qquad \text{(Eq. 3)}$$

where Q is the rate of the blood flow (ml/min/g of tissue); F_r is the rate of the reference sample (0.49 ml/min); i_x is the radioactivity of the piece of the tail; and i_γ is the radioactivity of the reference blood sample (gram).

Each piece of the tail (2 cm) was calculated as percent of the total tail length. In the calculations a mean value of the blood flow in the tail was used: The blood flow of each piece of the tail was summarized and divided by the number of tail pieces, and the mean value of all the rat tails was calculated. The value was transformed from milliliter per minute per gram of tail tissue to milliliter per minute per milliliter of tail tissue after correction for the density of the tail tissue.

Comparison of Calculated and Measured Tail Concentrations of Antipyrine—Concentrations of antipyrine in the tail blood were calculated by means of a formula (15) based on Fick's principle. When a freely diffusible biologically inert tracer substance is carried to a tissue by the blood, the concentration in the tissue (C_i) at time T, is determined by the arterial concentration (C_a), the tissue–blood partition coefficient (λ_i), and blood flow per unit mass of tissue volume (F_i/V_i):

$$C_i\,(T) = \lambda_i k_i \, e^{-k_i T} \int_0^T C_a e^{kt} dt \qquad \text{(Eq. 4)}$$

where $k_i = m_i F_i/\lambda_i V_i$ and m_i is the diffusion coefficient for the substance. Equation 4 was used to calculate the concentration of antipyrine in the rat tail. The following values were used for the calculation: It is assumed that the diffusion of antipyrine does not limit tissue uptake and accordingly the coefficient of diffusion (m_i) is 1 (15). The partition coefficient of antipyrine between tissue and blood (λ_i) is 1, as taken from investigations of antipyrine distribution between brain–blood (16) and kidney–blood (17). Accordingly, it is assumed that the antipyrine concentration of tail tissue is equal to the concentration of tail blood.

Experimental Design—Blood flow to the tail was measured in each unanesthetized rat placed in restraining cages at ambient temperatures of 22 and 37°. The tail was placed in a water bath of 37°, and after 30 min ~300,000 microspheres labeled with cesium 141 were infused. The tail was then transferred to a water bath of 22°, and after 30 min an approximately equal amount of microspheres labeled with strontium 85 were infused. In this way the rats served as their own controls. Room temperature was kept constant at 22°.

Experiments—The microspheres[7] (15 ± 1 μm, density 1.3) were dissolved in sucrose/epichlorohydrin polymer[8] and sterile 0.9% saline to

[1] Møllegaard Hansens Avlslaboratorier, Ejby, Denmark.
[2] A/S Mekos, Helsingborg, Sweden.
[3] PE 50, Intramedic Clay Adams, Parsippany, N.J.
[4] Manufactured in this laboratory. The volume of the cage can be varied to fit rats of different size so as to allow movement back and forth but no rotation.
[5] [N-^{14}C]methyl antipyrine, New England Nuclear Corp., Boston, Mass.
[6] [^{3}H]acetaminophen, generally labeled, New England Nuclear Corp., Boston, Mass.
[7] Microspheres labeled with either strontium 85 or cesium 141, 3M Company, St. Paul, Minn.
[8] 10% Ficoll-70, average molecular weight 70,000, Pharmacia Fine Chemicals, Uppsala, Sweden.

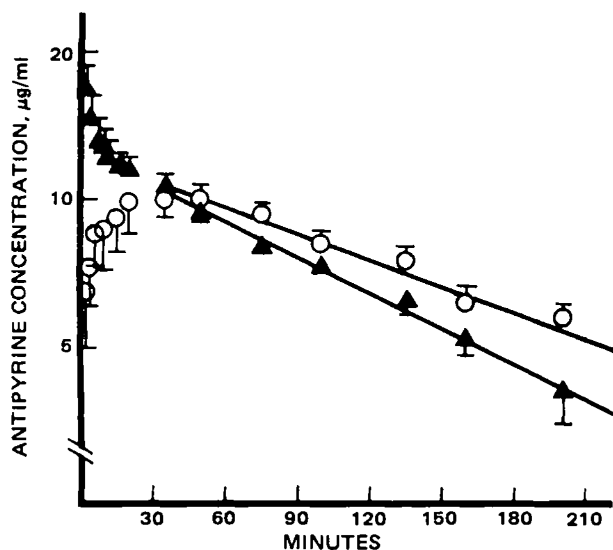

Figure 1—*Concentrations of antipyrine after administration of 15 mg/kg iv in blood sampled simultaneously from the cut end of the tail (O) and from a cannulated inguinal artery (▲). Values are mean ± SEM (n = 6).*

a concentration of ~6 × 10^5 microspheres/ml. Prior to microsphere infusion, a pump connected with the femoral artery catheter was started, sampling arterial blood at a constant rate of 0.49 ml/min for 2 min. This reference blood sample was used to calculate the blood flow to different organs according to a previous report (14). The solution of microspheres (0.5 ml) was infused *via* the cannula in the carotid artery within 15 sec, including flushing of the catheter with 0.3 ml of saline. After the last microsphere injection, rats were sacrificed by an intravenous injection of saturated potassium chloride. The tails were divided into pieces of ~2 cm, weighed, and placed in separate counting vials. The radioactivity was counted in a gamma liquid scintilation counter. The activity of the reference blood samples and both of the kidneys were also counted.

Statistical analysis—The results are given as means ±*SEM* and evaluated by paired Student's *t* test. Values for $p < 0.05$ were considered significant.

RESULTS

Pharmacokinetic Experiments—The time course of arterial and tail blood concentrations of both antipyrine and acetaminophen were widely different, and differences were also seen between the two drugs (Figs. 1 and 2). Individual tail concentration curves for both drugs were greatly variable, as indicated by the high standard error of the mean in the early phases of the experiment. After intravenous infusion of antipyrine, arterial concentrations showed the expected biexponential decline. Tail concentrations, however, increased until 40–60 min after the end of infusion. Acetaminophen concentrations showed a biexponential decline both in arterial and tail blood. Within the first 6 min after antipyrine infusion, the mean ratio of arterial to tail concentration decreased from 3 to 1.5. Mean arterial values were significantly different from mean tail values 0–15 min and >75 min after infusion ($p < 0.05$). Crossing of the curves took place 40–60 min after infusion. After 180 min the ratio of arterial to tail concentration was further decreased to 0.67. Corresponding values for acetaminophen concentrations for the first 6 min were 1.5, and after 60 min 0.5. Crossing of the acetaminophen curves was seen 12–20 min after infusion. All mean arterial concentrations of acetaminophen measured were significantly different from corresponding tail concentrations ($p < 0.05$).

Comparison of data based on arterial and tail concentrations of antipyrine showed statistically significant differences ($p < 0.05$) in the following pharmacokinetic variables (Table I). The biological half-life ($t_{1/2}\beta$) calculated from tail concentrations was higher by 47.5% compared with calculations based on arterial data, and the apparent elimination rate constant β was lower by 24%. Total clearance for antipyrine based on tail data was lower by 29.5% compared with arterial data. Significant changes were also found for the calculated variables of acetaminophen (Table I). The value for $t_{1/2}\beta$ based on tail blood data was higher by 46% compared with arterial blood data and was reduced by 46% for tail blood

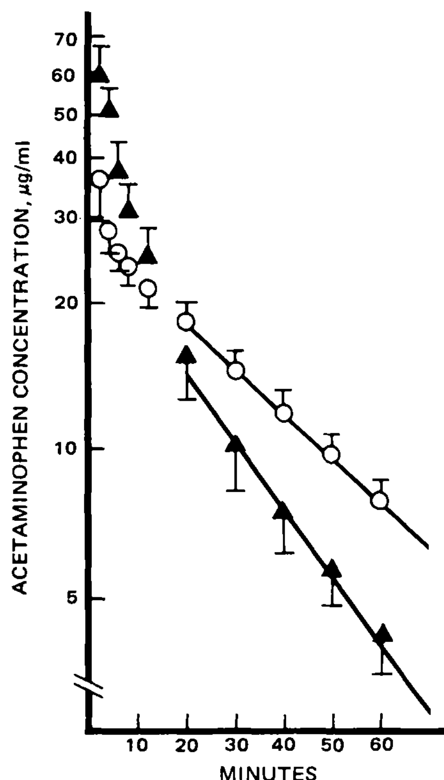

Figure 2—*Concentrations of acetaminophen after administration of 15 mg/kg iv in blood sampled simultaneously from the cut end of the tail (O) and from a cannulated inguinal artery (▲). Values are mean ± SEM (n = 5).*

data. In addition, the intercept of the extrapolated β phase, B, was lower by 11% for the tail blood data compared with arterial blood data. Also, the apparent volume of distribution, V_β, was significantly smaller with tail blood concentrations (35%).

Determination of Tail Blood Flow—The mean tail blood flow for five rats is shown as a function of the length of the tail in Fig. 3. The flow at an environmental temperature of 37° was significantly larger than the flow at 22° ($p < 0.05$).

Comparison of Calculated and Measured Tail Concentrations of Antipyrine—The mean blood flow of the tail of individual rats was calculated after correction for the density of the tail tissue (1.1 g/ml)[9]. The mean tail flow of five rats was then calculated. Tail blood flow at 37° was 5.0 ± 2.2 × 10^{-2} ml/min/ml tail tissue and at 22° 2.05 ± 0.93 × 10^{-2} ml/min/ml tail tissue. Figure 4 shows mean values of antipyrine arterial concentrations ($n = 3$) and calculated tail antipyrine concentrations at 37 and 22°. The time course of the calculated antipyrine tail concentration (Fig. 4) was grossly similar to actually measured concentrations presented in Fig. 1. Like measured tail concentrations, calculated tail concentrations were less than half the arterial concentration values at the earliest time points. Crossing of the lines likewise was seen 40–60 min after infusion.

DISCUSSION

The present study demonstrates that calculations based on drug concentration data from tail blood may give rise to pharmacokinetic values significantly different from values calculated from arterial blood samples. If it is assumed that rat arterial concentrations represent true kinetics, the use of rat tail concentrations may introduce significant errors in the pharmacokinetic calculations.

Although the pharmacokinetic data of antipyrine calculated from arterial concentrations are in accordance with values reported from other laboratories using different techniques (18, 19), it would be very difficult to prove that arterial blood data represent true kinetics in the rat. Serial sampling of arterial blood from individual rats will always require some kind of cannulation procedure. The stress imposed by the surgical pro-

[9] Obtained by dividing tail weights ($n = 3$) by the volume of water displaced.

Table I—Antipyrine and Acetaminophen Kinetics Based on Arterial and Tail Data [a]

	Site of Blood Sampling	Cl_T, ml min^{-1} kg^{-1}	β, 10^{-3} min^{-1}	$t_{1/2}\beta$, min	$\surd\beta$, liter kg^{-1}
Antipyrine	Artery	6.4 ± 0.4	5.4 ± 0.4	131 ± 11	1.18 ± 0.06
	Tail	4.5 ± 0.6[b]	4.1 ± 0.6[b]	194 ± 37[b]	1.15 ± 0.13
Acetaminophen	Artery	12.5 ± 1.0	33.0 ± 2.5	21.6 ± 1.8	0.39 ± 0.04
	Tail	13.1 ± 2.0	21.2 ± 1.4[b]	33.4 ± 2.6[b]	0.60 ± 0.09[b]

[a] The pharmacokinetic variables were calculated from arterial and tail concentration data in blood samples drawn simultaneously (see *Experimental*). Values are means ± *SEM* (n = 6, antipyrine; n = 5, acetaminophen). [b] Significantly different from arterial data ($p < 0.05$).

cedure (20) may influence significantly drug kinetics (21–23). The fact that antipyrine and acetaminophen kinetics were not reproducible in untreated animals over a short period of time (3) probably reflects that at least some aspects of normal physiology with relevance to pharmacokinetics are difficult to maintain even for short periods in cannulated rats.

There is only sparse information in the literature with respect to rat tail flow. Therefore, the rat tail flow was investigated by means of the radioactive microsphere technique for determination of regional blood flow (13). The following requirements must be fulfilled for the method to provide an exact measure of regional blood flow: First, there should be homogeneous mixing of microspheres with the arterial blood. According to previous reports (24, 25) this need will be met if the left ventricle is used as the site of injection. Verification of uniform mixing in the left ventricle can be obtained by measuring the left and right kidney blood flow (milliliters per minute per gram). Second, the microspheres must not recirculate. Spheres of 15 μm are shown to be trapped precapillary in the first circulation with only 1–2% ending in the lung capillaries of the rat, hence, they satisfy this requirement (26). Third, blood flow must not be affected by microspheres. Repeated microsphere injections in the same rats using different labels should not give significant changes in cardiac output fractions. Using 15-μm spheres, unchanged distribution was found (27) after the second microsphere injection in rats. In the present investigation, spheres of this size were used, and the present dose of 15-μm spheres have been shown not to interfere with this hemodynamic variable in the rat (14. 28). Fourth, the number of microspheres injected should give at least 400 in each tissue sample to avoid random error (24).

A previous study (4) reported flow values with a less sensitive technique at 17 and 33° that were two- and eightfold higher than the present values measured at 22 and 37°, respectively. However, the data presented here demonstrate that the tail blood flow is very low and represents only a minor fraction of the cardiac output. By comparison, rat tail flow in this study was only 8.8 and 0.4% of the rat liver and kidney flow values reported in a study using the same microsphere technique (28). This explains why tail concentrations lag behind arterial concentrations when the latter change rapidly. If tail flow is increased, for instance by elevation of tail temperature, the lag time will be reduced as shown in Fig. 4.

The close correspondence of the observed and calculated antipyrine tail concentrations strongly suggests that the assumption made for the theoretical relationship between tail concentrations, arterial concentrations, and time were correct; *i.e.*, the coefficient of diffusion (m_i) and the partition coefficient of antipyrine between tissue and blood (λ_i) are 1 (15–17), and accordingly, the organ blood flow becomes the rate-limiting factor. The different ratios observed between arterial and tail concentrations for antipyrine and acetaminophen (Figs. 1 and 2) could reflect that distribution properties of acetaminophen influence tail concentrations of the drug to a greater extent than the corresponding properties of antipyrine.

The effect of temperature changes on tail flow shows that drug kinetics based on tail concentration data may significantly reflect changes in the environment in addition to changes caused by drug distribution and elimination processes. Furthermore, intersubject differences in sensitivity of the tail to environmental factors may explain the great variation in the shape of antipyrine and acetaminophen tail concentration curves shortly after drug injection.

The variation of tail kinetics between individual rats contrasts sharply with the lack of variation observed within individual animals when antipyrine kinetics were retested after intervals of several weeks using blood samples taken >60 min after antipyrine infusion (9, 11, 29).

These observations suggest that by strict standardization of the experimental conditions, tail flow can be reproducible from one experiment

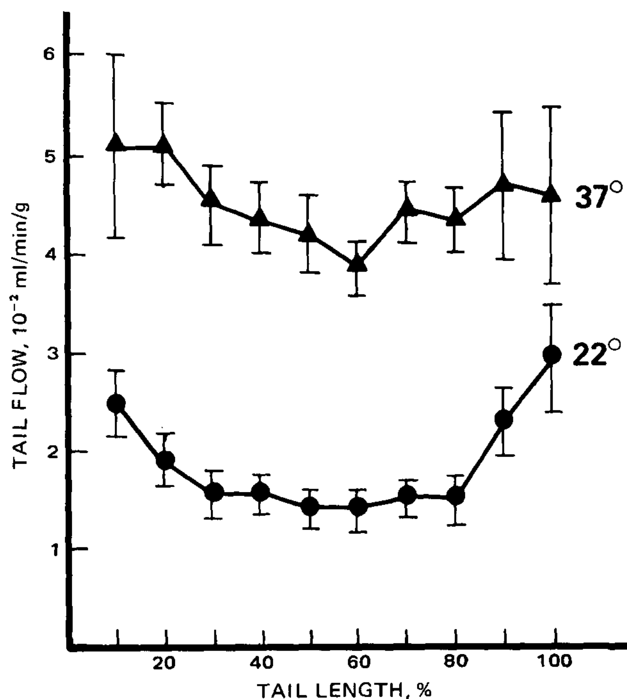

Figure 3—*Blood flow through the rat tail measured by means of microsphere technique at environmental temperatures of 22 (●) and 37° (▲). Values are mean ± SEM (n = 5). From left to right is from the tip to root of the tail.*

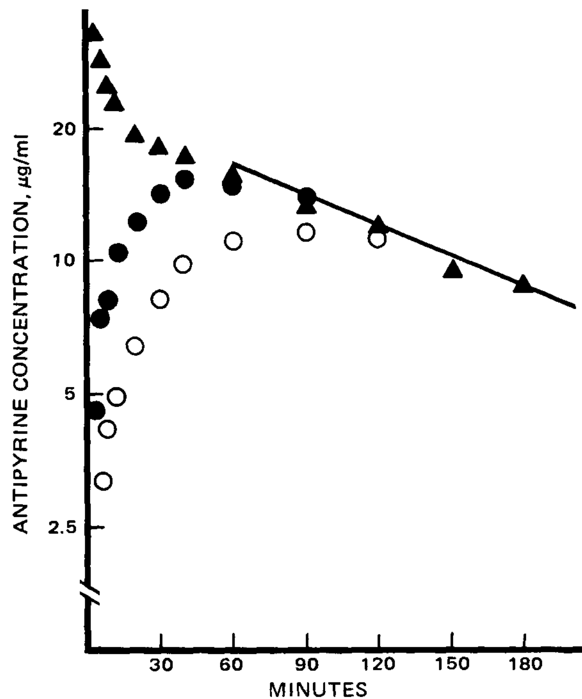

Figure 4—*Concentrations of antipyrine after administration of 15 mg/kg iv in blood sampled from a cannulated inguinal artery (▲) and calculated concentrations of antipyrine in tail tissue at 22° (○) and at 37° (●) (n = 3).*

to another in untreated rats. Problems arise, however, when the method is used to detect changes in pharmacokinetics by some experimental factors. Although the tail method detected changes in antipyrine kinetics by short-term, low-dose treatment of phenobarbital (11) and long-term treatment with ethanol (9), interpretation of data is obscured by possible influences of the treatment on tail blood flow and tail antipyrine distribution. Further investigations of such effects on drug kinetics, therefore, should include *in vitro* studies (9) or kinetic studies with the use of arterial blood samples.

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Evaluation of Chemical Analysis for the Determination of Solasodine in *Solanum Laciniatum*

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Abstract □ A detailed study of a method for solasodine analysis has been carried out and the suitability of chemical analysis for solasodine determination in plant material evaluated. A number of problems with the analytical isolation of solasodine and its subsequent colorimetric determination have been highlighted: oven drying of plant material >100° leads to solasodine loss; cell disruption of the dry plant material is required if complete and rapid extraction of solasodine is to take place; hydrolysis of plant extract residues in >1 *N* acid leads to solasodine loss; the colorimetric procedure is more temperamental than past methods have indicated, especially with regard to the specificity of the reaction and the instability of the complex.

Keyphrases □ Solasodine—steroidal alkaloid from *Solanum laciniatum*, colorimetric determination by evaluation of chemical analysis □ Steroids—colorimetric determination of the alkaloid, solasodine, in *Solanum laciniatum* □ Colorimetry—determination of solasodine in *Solanum laciniatum*

Since problems arose 6 or 7 years ago with the supply, cost, and steroid content of *Dioscorea*, the source of diosgenin for steroid drug production, there has been renewed interest in alternative raw materials, including solasodine from plants of the genus *Solanum*. This steroidal alkaloid occurs in *S. aviculare* and *S. laciniatum* as the glycosides, solasonine and solamargine.

As part of a study into the production of solasodine from *Solanum* plant material, chemical analysis was considered for the determination of solasodine and its related species. Several important observations were made in the course of this investigation concerning both the isolation of solasodine from the plant (sample preparation, extraction, and hydrolysis) and the subsequent determination of solasodine using colorimetry. Chemical analysis was found not to be suitable for this study, and subsequently, a procedure using high-pressure liquid chromatography was developed (1). However, chemical analysis is suitable for certain purposes and has the advantage of not requiring expensive equipment. Matters concerning the isolation of solasodine from the plant material are important in solasodine analysis in general (including instrumental analysis) and in commercial solasodine production.

Previous methods for chemical analysis of solasodine

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