Table I—Pharmacokinetic Parameters of Procainamide and *N*-Acetylprocainamide

Parameter	Rats				Humans	
	Our Study		Ref. 10			
	Procainamide	*N*-Acetylprocainamide	Procainamide	*N*-Acetylprocainamide	Procainamide[a]	*N*-Acetylprocainamide[b]
Elimination half-life ($t_{1/2}$), hr	0.66	2.13	0.92	0.85	3.5	6.0
Plasma clearance, ml/min/kg	80.7	20.7	64.0	22.0	7.99	3.09
Apparent volume of distribution, liters/kg	4.60	3.82	4.92	1.64	1.91	1.61

[a] From Ref. 7. [b] From Ref. 8.

gression analysis program available in a statistical analysis system package[2].

The biological half-life ranged from 0.54 to 0.83 hr for procainamide and from 1.72 to 2.92 hr for *N*-acetylprocainamide. The ratio of the biological half-life of *N*-acetylprocainamide to that of procainamide in the same rat averaged 3.3. Table I summarizes the values of the pharmacokinetic parameters obtained by Schneck *et al.* (10), those obtained in our study, and values reported for normal subjects.

Determination of the biological half-lives of procainamide and *N*-acetylprocainamide in the same animal, with sufficient time for elimination of the drugs from the body, allowed a more meaningful comparison of the pharmacokinetic parameters of the two drugs. The discrepancy between these results and those reported by Schneck *et al.* (10) may be due to the study design and to the method of determination of pharmacokinetic parameters. Schneck *et al.* sacrificed groups of rats and followed the blood sampling for 4 hr, which appears to be inadequate for *N*-acetylprocainamide. In our study, a distribution phase of ~1 hr was noticed when *N*-acetylprocainamide was administered intravenously. Furthermore, Schneck *et al.* (10) calculated the volume of distribution by dividing the total body clearance by the elimination rate constant, which obviously results in an underestimated value (Table I).

The results of our study were qualitatively similar to those observed in normal human subjects. The ratio of the half-lives, volumes of distribution, and body clearances of procainamide and its metabolite in these two species are similar. These results have important implications in interpretations of metabolism data from acute and chronic toxicity studies.

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Assignment of ^{13}C-NMR Spectra of Strychnine and Brucine

Keyphrases □ Alkaloids—strychnine and brucine, ^{13}C-NMR spectral analyses, chemical shifts assigned □ ^{13}C-NMR spectroscopy—brucine and strychnine, chemical shifts assigned □ Brucine—^{13}C-NMR spectral analysis, chemical shifts assigned □ Strychnine—^{13}C-NMR spectral analysis, chemical shifts assigned

To the Editor:

Singh *et al.* (1) recently reported on the ^{13}C-NMR spectroscopy of the alkaloids strychnine (I) and brucine (II). In this communication, I shall comment on the spectral data and assignments reported by these authors.

Wehrli (2, 3) previously reported on the ^{13}C-NMR spectrum of brucine. The complete assignment of the brucine spectrum (3) was accomplished by using common

Table I—Assignment of ^{13}C-NMR Spectra of Strychnine

Carbon	Ref. 1	Ref. 4	Ref. 5	Ref. 7	Ref. 6	Ref. 8
1	124.2 d	124.25 d	124.12 d	124.99 d	122.3 d	121.9 d
2	122.3 d	122.31 d	122.17 d	122.67 d	124.3 d	123.8 d
3	128.6 d	128.62 d	128.41 d	128.61 d	128.6 d	128.1 d
4	116.3 d	116.24 d	116.12 d	116.52 d	116.2 d	115.8 d
5	142.3 s	142.21 s	142.21 s	142.21 s	142.2 s	141.8 s
6	132.7 s	132.52 s	132.75 s	132.91 s	132.6 s	132.4 s
7	52.0	51.93 s	51.89 s	52.33 s	51.9 s	51.7 s
8	60.2 d	60.06 d	60.08 d	60.42 d	60.1 d	59.9[a] d
10	169.3 s	169.39 s	169.18 s	170.31 s	169.4 s	168.8 s
11	48.2	42.34[a] t	50.28 t	42.46 t	42.3[a] t	42.2 t
12	77.2 d	77.53 d	77.54 d	77.73 d	77.5 d	77.3 d
13	42.9 d	48.17 d	48.18 d	48.44 d	48.2 d	48.0 d
14	42.5 d	31.54 d	31.60 d	31.83 d	31.5 d	31.4 d
15	31.7 t	26.81 t	26.87 t	26.92 t	26.8 t	26.7 t
16	60.2 d	60.06 d	60.08 d	60.42 d	60.1 d	59.8[a] d
17	26.9 t	42.83[a] t	42.43 t	43.05 t	42.8 t	42.6 t
18	52.6	50.23 t	52.67 t	50.49 t	50.2 t	50.1 t
20	50.4	52.65 t	42.87 t	52.76 t	52.7 t	52.4 t
21	140.5 s	140.27 s	140.56 s	140.04 s	140.3 s	140.2 s
22	127.5 d	127.65 d	127.09 d	128.99 d	127.7 d	125.8 d
23	64.6 t	64.55 t	64.57 t	64.84 t	64.6 t	64.3 t

[a] Interchangeable.

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Table II—Assignment of ^{13}C-NMR Spectrum of Brucine

Carbon	Ref. 1	Ref. 2	Ref. 3	Ref. 4	Ref. 5	Ref. 6	Ref. 8
1	105.6 d		105.88 d	105.68 d	105.68 d	105.7 d	105.1 d
2	146.2 s	149.13/146.14	149.13/146.14 s	146.24 s	146.11 s	146.2 s	145.6 s
3	149.2 s	146.14/149.13	146.14/149.13 s	149.25 s	149.13 s	149.3 s	148.5 s
4	101.1 d		101.08 d	101.07 d	101.00 d	101.1 d	100.5 d
5	136.0 s	135.97	135.97 s	136.02 s	135.91 s	136.0 s	135.3 s
6	123.6 s	123.46	123.46 s	123.64 s	123.48 s	123.6 s	122.9 s
7	51.8		51.83 s	51.93 s	51.84 s	51.9 s	51.4 s
8	59.9 d		60.22 d	60.30 d	59.84 d	60.3 d	59.8[a] d
10	168.9 s	168.64	168.64 s	168.90 s	168.78 s	168.9 s	168.2 s
11	48.2		42.20 t	42.34 t	50.09 t	42.3 5	41.8 t
12	77.3 d		77.63 d	77.78 d	77.64 d	77.8 d	77.1 d
13	42.4 d		42.15 d	48.29 d	48.18 d	48.3 d	47.7 d
14	42.4 d		31.51 d	31.54 d	31.50 d	31.5 d	31.1 d
15	31.6 t		26.73 t	26.81 t	26.73 t	26.8 t	26.3 t
16	60.3 d		59.82 d	59.94 d	60.28 d	59.9 d	59.3[a] d
17	26.8 t		42.35 t	42.34 t	42.28 t	42.3 t	41.9 t
18	52.6		50.04 t	50.11 t	52.57 t	50.1 t	49.6 t
20	50.2		52.57 t	52.66 t	42.28 t	52.7 t	52.1 t
21	140.6 s	140.29	140.29 s	140.63 s	140.40 s	140.6 s	139.8 s
22	127.1 d		127.14 d	127.16 d	127.14 d	127.2 d	126.7 d
23	64.6 t		64.44 t	64.55 t	64.47 t	64.6 t	64.0 t
	56.4 q		56.45 q	56.42 q	56.43 q	56.4 q	55.7 q
	56.1 q		56.05 q	56.18 q	56.08 q	56.2 q	56.0 q

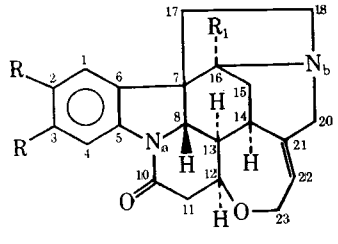
[a] Interchangeable.

shift rules, off-resonance spectral data, and spin-lattice relaxation time data of the low-field quaternary carbons. Verpoorte and Baerheim Svendsen (4) also reported on the ^{13}C-NMR spectroscopy of strychnine, brucine, and alcuronium, and Srinivasan and Lichter (5) reported on the ^{13}C-NMR spectroscopy of strychnine and brucine.

My extended studies of ^{13}C-NMR spectroscopy of a series of Strychnos alkaloids (6) showed that several assignments made by Srinivasan and Lichter were erroneous. Furthermore, this study (6) made it necessary to reverse my earlier assignment of the signals for C-1 and C-2 in strychnine. Leung and Jones (7) reported their investigation of the ^{13}C-NMR spectroscopy of some Strychnos alkaloids, and their assignment for the spectrum of strychnine was the same (except for C-1 and C-2) as in my second paper (6). Wenkert *et al.* (8) also reported on the ^{13}C-NMR spectroscopy of a series of Strychnos alkaloids. Their assignment of the strychnine spectrum was similar. All spectral data of Refs. 1–8 are summarized in Tables I and II.

If the results reported by Singh *et al.* (1) are compared with those reported by the other investigators, a great deal of discrepancy is observed. The multiplicity for some signals was not reported by Singh *et al.* [C-7 (52.0), C-11 (48.2), C-18 (52.8), and C-20 (50.4) in strychnine]; for other signals [*i.e.*, 42.5 (d), 42.9 (d), and 31.7 (t) in strychnine], the multiplicities were different from those reported in the other studies [42.3 (t), 42.8 (t), and 31.5 (d)] (2–8). In the off-resonance decoupled spectra of strychnine and brucine, not all of the signals show neat first-order splitting patterns. Also, second-order patterns are observed for some signals (9). This finding is readily understood if the PMR spectrum of strychnine is considered, where several methylene groups have the attached *geminal* protons at rather different shifts (10). However, by using different decoupling frequencies in the off-resonance decoupled spectra, the multiplicity of the signals can be established (11). The erroneous interpretation of the multiplicity of some signals necessarily leads to an erroneous assignment of these signals.

Furthermore, the assignment of C-17 by Singh *et al.* (1) is different from the assignments reported by other investigators. However, the assignment of the 42.8-ppm signal to this carbon is confirmed by comparison of the spectra of strychnine and pseudostrychnine (6). In the spectrum of the latter compound, the 26.8-ppm signal is shifted downfield considerably, which makes assignment of this signal to C-15 plausible. These arguments and the data given in Tables I and II indicate that the assignment of the strychnine and brucine spectra according to Singh *et al.* (1) is inaccurate. The assignment of the ^{13}C-NMR spectra of strychnine and brucine as reported by Wehrli (3), Verpoorte *et al.* (6), and Wenkert *et al.* (8) is correct.



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II: R = OCH_3, R_1 = H

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Potential Effect of Early Blood Sampling Schedule on Calculated Pharmacokinetic Parameters of Drugs after Intravenous Administration

Keyphrases □ Pharmacokinetics—effect of blood sampling time on calculated parameters after intravenous administration □ Volume of distribution—effect of blood sampling time on values calculated after intravenous administration □ Blood sampling time—effect on calculated pharmacokinetic parameters after intravenous administration

To the Editor:

The disposition of a drug in the body often can be studied best by intravenous administration (1–5). Differences in experimental methodology are known to affect the results of a pharmacokinetic study. For example, an insufficient sampling period or the lack of a sensitive assay may result in the underestimation of terminal biological half-lives of drugs (4–9). A nonspecific assay can cause overestimation of the plasma area under the curve and underestimation of the total plasma clearance (10). Sampling devices (11) and blood containers (12) might also affect assay results and, hence, the calculated pharmacokinetic parameters. Marked differences in protein binding (possibly also the concentration) of several drugs and bilirubin in serum and heparinized plasma were demonstrated (13). Differences in the curve-fitting technique also might affect the characterization of pharmacokinetic properties (4, 5, 14, 15).

In conventional pharmacokinetic studies, plasma concentration profiles between the beginning of intravenous injection or infusion (*i.e.*, time zero) and the time of the first blood sample collected usually are extrapolated or predicted based on plasma level data obtained at later times. The accuracy of such an extrapolation method has been questioned (16, 17). Marked underestimations of extrapolated plasma concentrations at time zero and probably of extrapolated plasma areas under the curve between time zero and the first sampling time (15 min) after rapid intravenous injection of sulfamethizole to five dogs were found when a constant blood withdrawal device and the conventional multiple blood sampling techniques were employed simultaneously (16). Disposition kinetics of a drug within the first few minutes after intravenous injection are often more complicated than is commonly recognized (17). For example, the true peak plasma concentration might be several times higher than the extrapolated concentration (17). Furthermore, the peak concentration in humans might occur 0.5–2 min after the end of dosing; at time zero, the true plasma concentration at the normal sampling site should always be zero and not the extrapolated zero-time value.

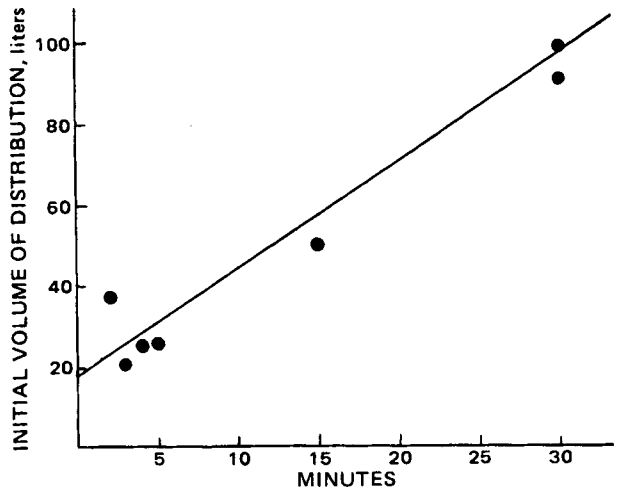

Figure 1—*Correlation between the average initial volume of distribution, V_c, and the time of the first blood sampling after an intravenous dose of digoxin in seven studies on humans with normal renal function; $V_c = 17.4 + 2.53t$ ($r^2 = 0.9452$).*

This review indicates that the early blood sampling schedule used in an intravenous study sometimes might significantly affect the obtained disposition function and, hence, the resultant multicompartmental modeling. In a recent study on quinidine, it was stated that the sampling pattern selected in a study also influences the choice of a model (15). Although it was not elaborated further, this statement is consistent with the contention of the possible early sampling schedule effect.

The literature often shows marked variability in the mean initial volume of distribution or mean volume of the central compartment reported in different intravenous studies on the same drug in similar subjects or patients. Although many factors might account for some of the differences, there often is a general pattern that the mean initial volume of distribution is smaller if the first blood sample is collected earlier in a study. The purpose of this communication is to point out this apparent trend using digoxin, gentamicin, and thiopental as examples.

Mean apparent initial volumes of distribution of digoxin reported or analyzed from six studies (18–23) on healthy adults with normal renal function are used for comparison. Subject characteristics, the duration of intravenous administration, the time of the first blood sampling, and the calculated mean volume value for each study are summarized in Table I. An apparent linear relationship between the mean initial volume of distribution and the time of the first blood sample was found (Fig. 1). The lowest volume, 20 liters, and the highest volume, 99 liters, were obtained from studies with the first blood sample collected at 3 and 30 min, respectively.

Although it is more meaningful to compare the volume of distribution in terms of volume per unit of body weight, this comparison usually is not possible since information on body weight was reported only in one study (21). For that study, the value (24.5 liters) reported in Table I was corrected for a 70-kg body weight. Since these studies involved normal adult subjects with a relatively narrow age range (no age information in Ref. 19), it is unlikely that body weight is a major factor for the marked variation in

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