measurement time of one sample in the electron-capture GLC (~15 min) is longer than that in the mass fragmentography. The mass fragmentography appears to be more readily adaptable to large numbers of samples.

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COMMUNICATIONS

Pharmacokinetics of Drugs in Blood III: Metabolism of Procainamide and Storage Effect of Blood Samples

Keyphrases □ Pharmacokinetics—procainamide metabolism, storage effect of blood samples, *N*-acetylprocainamide □ Procainamide metabolism—pharmacokinetics, storage effect of blood samples, *N*-acetylprocainamide

To the Editor:

The distribution kinetics and metabolism of drugs in blood are of importance in pharmacokinetic studies. For instance, if a drug equilibrates slowly between plasma and blood cells, the time elapsed between collection and centrifugation of a blood sample could have a significant effect on the plasma concentration measured, which might differ considerably from the true *in vivo* concentration. This has been recently shown with gentamicin and furosemide (1, 2). A similar problem might also be anticipated if a drug undergoes *in vitro* metabolism in the blood. The above phenomenon has been referred to as the storage effect of blood (1, 2). This communication reports our preliminary studies on the metabolism of procainamide in blood and the storage effect of blood samples for procainamide and its metabolites.

Freshly withdrawn[1], heparinized control blood from five healthy subjects, aged 25–41 years, was used. None of the subjects received any medication for at least 1 month prior to the study. The whole blood (10 ml) in test tubes[2] from each subject was spiked with procainamide[3] stock solution (2 mg/ml of free base) to yield a concentration of 20 μg/ml. After manually mixing for 30 sec, each blood sample was quickly divided (1.0 ml) into 10 vials. The sealed vials were then stored (time zero) in a refrigerator (5°). To prevent any interaction between the drug and the stopper, the screw caps of the test tubes and vials used were all lined with aluminum foil. Similar preparations were carried out with blood samples kept at ambient temperature (25 ± 1.0°). These temperatures were chosen in order to simulate common procedures for handling blood samples. The vials were removed and centrifuged immediately at various times up to 48 hr. All the plasma samples were frozen until analyzed for procainamide and its metabolite, *N*-acetylprocainamide, using a modified high-performance liquid chromatographic (HPLC) method which was developed earlier in this laboratory (3).

Briefly, the assay involved the addition of 0.25 ml acetonitrile to 0.1 ml of plasma sample in a test tube[2]. After vortex-mixing and centrifugation, 20 μl of the supernatant solution was injected directly onto the column. The instrumentation consisted of a solvent delivery pump[4], a syringe-loading sample injector[5], a cation exchange column[6], and a fixed-wavelength detector[7] with 254-nm filter. The mobile phase was made of 75% (v/v) 0.12 *M* ammonium phosphate acidified with phosphoric acid (0.2%) and 25% acetonitrile; the flow rate was 2.5 ml/min. By this method, the detection limit was 0.2 μg/ml for both procainamide and *N*-acetylprocainamide in plasma.

Figure 1 shows the typical plasma concentration profiles of procainamide from the above studies in three subjects. Assuming that procainamide initially was only confined to plasma, based on the individually determined hematocrits, one could estimate its concentrations from the three subjects to be 40, 40, and 35 μg/ml, respectively. However, the results (Fig. 1) show much lower concentrations (15.6–18.7 μg/ml) obtained immediately after spiking and brief mixing, indicating an initial rapid and extensive distribution of the drug to blood cells. Adsorption of the drug onto the glass tube could be ruled out, since there was no initial loss of procainamide from the whole blood in subsequent studies.

The plasma concentrations, in general, decreased with time (Fig. 1). However, fluctuations in the measured concentration were usually found during the initial 12 hr of

[1] Vacutainer, Division of Becton, Dickinson and Co., Rutherford, N.J.
[2] Fisher Scientific Co., Pittsburgh, Pa.
[3] Hydrochloride salt, E. R. Squibb & Sons, Inc., Princeton, N.J.
[4] Model 6000A, Waters Associates, Milford, Mass.
[5] Model 7125, Rheodyne, Berkeley, Calif.
[6] Partisil PXS 10/25 SCX, Whatman, Clifton, N.J.
[7] Model 440, Waters Associates, Milford, Mass.

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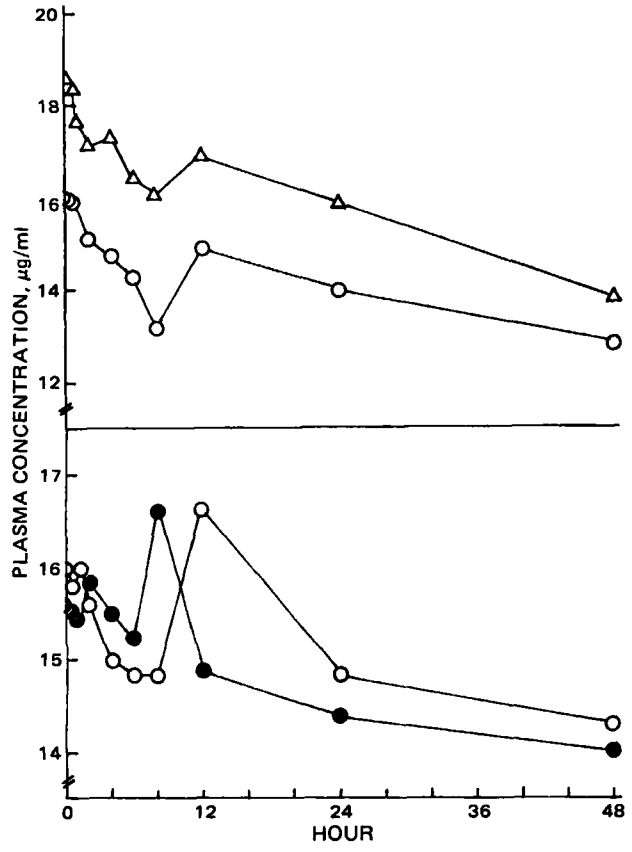

Figure 1—*Procainamide plasma concentration–time profiles from blood spiked with 20 μg/ml, and kept at ambient temperature (upper panel) or 5° (lower panel). Key: (○) subject 1; (●) subject 2; (△) subject 3.*

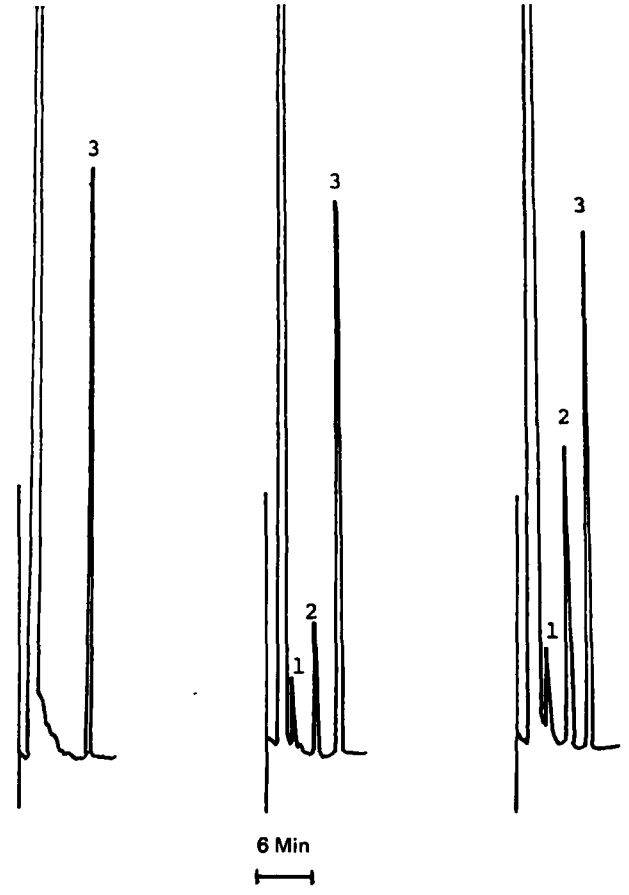

Figure 2—*Typical HPLC chromatograms from the blood of the first subject spiked with 20 μg/ml of procainamide and kept at ambient temperature for various periods of time: left, immediately after spiking (time zero); middle, 12 hr; right, 24 hr. Key: (1) unknown metabolite; (2) N-acetylprocainamide; (3) procainamide.*

study. Similar results were obtained when blood samples were spiked with 6 μg/ml of procainamide. These unusual distribution kinetics were observed in most of our studies either at 25 or 5°. It is felt that the above findings, especially those showing larger fluctuations, could not be an artifact, since the assays were conducted in duplicate and their coefficients of variation were <3.5%. In addition, simultaneous analysis of blood procainamide concentration in separate studies showed essentially a declining pattern, presumably due to metabolism of the drug in blood. At present, the cause or exact mechanism is unclear. Nevertheless, a possible reason might be the formation of Schiff bases between the drug and free fatty aldehydes on cell membranes, as proposed for gentamicin (1).

The formation of *N*-acetylprocainamide occurred in all the *in vitro* studies. This was shown by the appearance of peaks in the chromatograms (Fig. 2), with their retention time identical to that from the authentic samples. Typical time profiles of *N*-acetylprocainamide from subject 2 are depicted in Fig. 3. The *in vitro* formation of this metabolite in the present study appeared to be temperature dependent and to follow approximately a zero-order process, which could last up to 24 hr in some subjects. Based on the apparent linear portion of the curves (Fig. 3), the mean appearance rates of *N*-acetylprocainamide in plasma at 5 and 25° were calculated to be ~0.05 and 0.10 μg/ml/hr, respectively. It should be noted that although *N*-acetylation of procainamide has been found to take place in whole blood (4), the distribution kinetics and its implication were not reported.

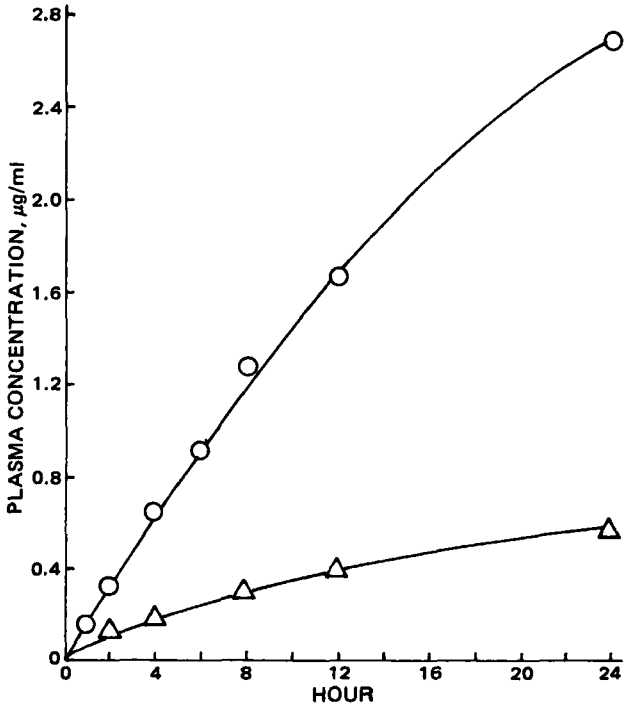

Figure 3—N-*Acetylprocainamide plasma concentration–time profiles from the blood of the second subject spiked with 20 μg/ml of procainamide and kept at 5° (△) and 25° (○).*

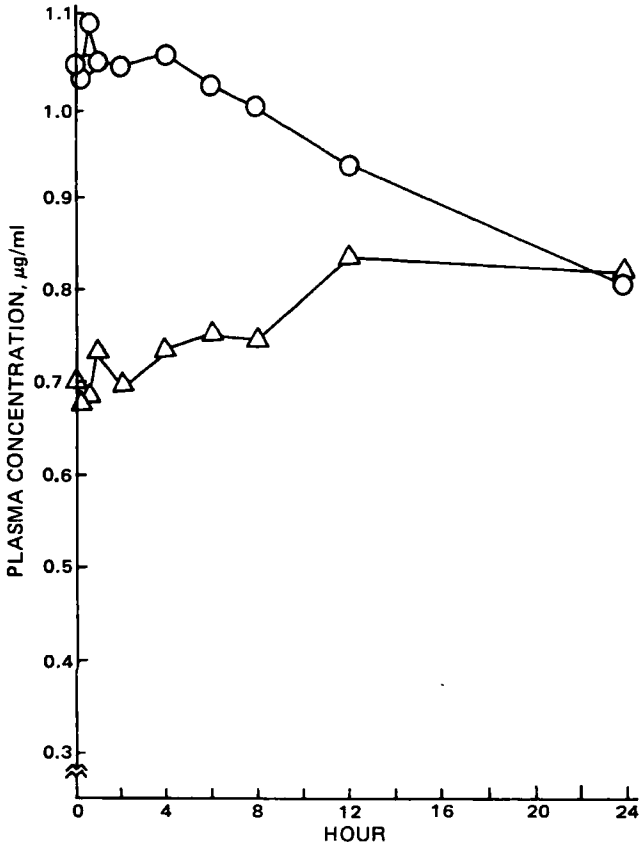

Figure 4—*Procainamide (○) and N-acetylprocainamide (△) plasma concentration–time profiles in a blood sample kept at ambient temperature.*

A second metabolite with shorter retention than *N*-acetylprocainamide (Fig. 2) was found in all chromatograms except those from samples obtained at earlier times. This unidentified metabolite was also found in many cases after the administration of procainamide to rabbits (5) and humans (6). Recent studies (7) have shown that desethyl procainamide and desethyl *N*-acetylprocainamide might be two additional metabolites of procainamide. However, identification of the unknown product was not made due to the lack of authentic samples. According to the relative peak heights, the appearance rate of this unidentified metabolite in plasma also followed a zero-order process in the first 12 hr of study.

To ascertain whether metabolism took place in plasma or blood cells, pooled plasma was spiked with procainamide to yield an initial concentration of 20 μg/ml and kept at ambient temperature for various periods of time. No metabolite formation could be found up to 48 hr, indicating that blood cells are the sole site of metabolism in whole blood. Since the plasma concentrations of procainamide were essentially identical during the study, no degradation of plasma samples could be assumed.

The results of the above *in vitro* studies suggest that the time between collection and centrifugation of a blood sample may have a considerable influence on the measured plasma levels of procainamide and its metabolites. This was supported by another experiment in which ~10 ml of blood was collected shortly before the next scheduled dose from an adult male patient on chronic oral procainamide therapy (Fig. 4). During the 24 hr of storage, the difference between the minimum and maximum plasma concentrations measured for procainamide was ~ 35% and that for *N*-acetylprocainamide was 24%. Similar effects also were observed from two rabbits, whose blood was collected in syringes[8] after intravenous dosing of procainamide.

In light of the results of this study, it appears that a prudent approach is to separate plasma as soon as the blood sample is collected; this might minimize the difference between the true *in vivo* plasma concentration and the measured *in vitro* concentration. More work is required in order to fully assess the potential significance of the present findings in the pharmacokinetic studies.

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Nonlinear Regression Approach for Determining Whether Absorption and Elimination Rate Constants are Equal in the One-Compartment Open Model with First-Order Processes

Keyphrases □ Pharmacokinetics—one-compartment open model, nonlinear regression analysis, absorption rate constants, elimination rate constants

To the Editor:

Recently Bialer reported a simple method for determining whether absorption and elimination rate constants are equal in the one-compartment open model with first-order processes (1). The basis for this method is that whenever the product of time of peak drug concentration (t_{max}) is equal to total area under curve (AUC) divided by the base of natural logarithm (e), the absorption rate constant (k_a) must be equal to the elimination rate con-

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