susceptibility disks were compared for performance. Grades A-740-E, A-470, and B-126 papers perform in the same manner. Grade B-676 paper appears to bind a high percentage of most antibiotic solutions applied to it. This binding is particularly pronounced with polypeptide and aminoglycoside drugs. Grade B-225 paper, on the other hand, frequently releases antibiotics at greater rates than the A-470 and A-740-E papers.

Analysis of slopes of dose-response lines did not show the same effects on classes of antibiotics from one paper to another. Again, the A-470 and A-740-E papers were generally equivalent for each drug. In a few instances, one or another of the production papers appeared to enhance the dose response compared to the slopes produced by A-740-E paper. Grade B-126 decreased the dose response to tetracycline. These types of differences in dose responses between papers nullify the concept of the design of the performance test used to certify production lots of the respective disks, rendering assay of these products quite inaccurate. Thus, it has been shown that, contrary to the intent of the Code of Federal Regulations, §460.1(a) (7), each grade of paper used in the manufacture of antibiotic susceptibility disks affects bacterial growth and/or the antibiotic in one or more instances.

Most potency assays are statistically valid. However, since sample disks of production papers frequently do not evoke the same response as a like quantity of antibiotic on a standard disk, universal agreement among control laboratories as to the type of paper to be used to prepare standard control disks is highly desirable. Such agreement could extend to the use of a common lot of paper by those laboratories and would greatly enhance the likelihood of interlaboratory concurrence in potency values of commercial lots of antibiotic disks.

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Microdetermination of Procainamide in Human Serum

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Abstract ☐ An electron-capture GLC method to measure procainamide (0.1–1 μg/sample) in human serum was developed. An internal standard, *p*-amino-*N*-[2-(dipropylamino)ethyl]benzamide, is added to the serum before the sample is alkalinized with pH 10.5 phosphate buffer and extracted with ethyl acetate. The ethyl acetate phase is evaporated to dryness, and the residue is reacted with pentafluoropropionic anhydride. *N*-Pentafluoropropionyl derivatives of the drug and the internal standard had retention times of 5 and 8 min, respectively, when chromatographed at 235° on a 1-m (4-mm i.d.) glass column packed with 5% OV-17 (carrier gas flow of 40 ml/min). The coefficient of variation was less than 5% for spiked standards. Furthermore, *N*-acetylprocainamide added to samples did not interfere. One hundred and eighty-six samples from 16 patients receiving procainamide intravenously were assayed by this GLC procedure and by a standard colorimetric method. Linear regression analysis yielded a correlation coefficient of 0.985 (slope, 1.040; intercept, 0.015).

Keyphrases ☐ Procainamide—electron-capture GLC analysis in human serum ☐ GLC, electron capture—analysis, procainamide in human serum ☐ Cardiac depressants—procainamide, electron-capture GLC analysis in human serum

Procainamide has been used clinically over the last 25 years for the prevention and treatment of ventricular arrhythmias. Several studies (1–4) demonstrating the utility of plasma procainamide concentrations in dosage regimen design have stimulated interest in procainamide assays.

The colorimetric technique of Mark *et al.* (5) is commonly used to determine procainamide in biological fluids. However, Gibson *et al.* (6) reported that *N*-acetylprocainamide, a major metabolite of procainamide in humans (7), may be hydrolyzed to procainamide under certain conditions. Recently, it was found (8) that the colorimetric method (5) as modified by Sitar *et al.* (9) did not result in the hydrolysis of *N*-acetylprocainamide. A specific flame-ionization detection GLC assay for procainamide was developed (10) and gave results similar to those of the colorimetric analysis.

Several GLC techniques (10–13) and a high-pressure liquid chromatographic (HPLC) procedure (14) were described for procainamide analysis, but these methods require at least 0.5–1 ml of serum. This report describes an electron-capture GLC determination of procainamide that requires as little as 0.1 ml of plasma. In addition, the results of this new procedure are compared to those obtained using the modified colorimetric procedure (9).

EXPERIMENTAL

Reagents—Stock solutions of procainamide hydrochloride[1], *N*-acetylprocainamide hydrochloride[2], and the internal standard, *p*-

[1] ICN Pharmaceuticals, Plainview, N.Y.
[2] Astra Pharmaceutical Products, Worcester, Mass.

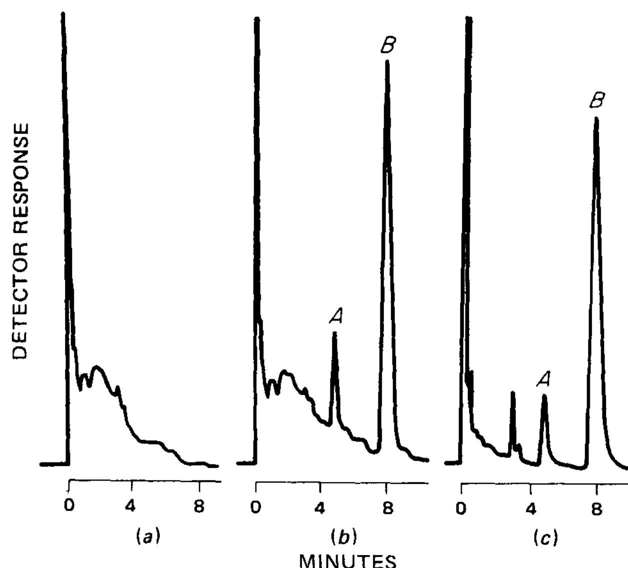

Figure 1—*Gas chromatogram of derivatized extract of 1 ml of pooled serum containing no procainamide or internal standard (*a*), 1 ml of pooled serum containing 0.1 μg of procainamide and 0.63 μg of internal standard (*b*), and 0.1 ml of pooled serum containing 0.1 μg of procainamide and 0.63 μg of internal standard (*c*). Key: A,* N-*pentafluoropropionyl procainamide; and B,* N-*pentafluoropropionyl derivative of the internal standard.*

amino-*N*-[2-(dipropylamino)ethyl]benzamide hydrochloride[3], were prepared in absolute methanol. The *N*-acetylprocainamide contained 1.1% procainamide. Pentafluoropropionic anhydride[4] was used as received. All other chemicals were reagent grade.

Phosphate buffer, pH 10.5, was prepared by dissolving 17.5 g of anhydrous dibasic potassium phosphate and 4 g of tribasic sodium phosphate dodecahydrate in a total volume of 100 ml of distilled, deionized water.

Extraction of Serum and Preparation of Serum Standards—To 15-ml culture tubes fitted with polytef-lined screw caps were added 1–10 μl (0.1–1 μg of base) of the procainamide stock solution and 7 μl (0.63 μg of base) of the internal standard stock solution. To determine if *N*-acetylprocainamide is hydrolyzed by extraction or derivatization, 100 μg of this metabolite was added to several tubes in place of procainamide. Methanol was removed by evaporation, and either 0.1 or 1 ml of pooled human serum was added. One gram of sodium chloride and 1 ml of pH 10.5 phosphate buffer (2 g of sodium chloride and 2 ml of buffer for 1-ml serum samples) were then added, and each sample was extracted with 5.5 ml of ethyl acetate.

Samples were shaken for 10 min at low speed on a reciprocating shaker[5] and then centrifuged for 10 min at 1500×*g*. Approximately 5 ml of the ethyl acetate was then transferred to a clean culture tube and evaporated to dryness at 40° in a stream of dry air. At this point, 100 μl of absolute methanol was added to each sample and then evaporated at 40°. This step was necessary to remove traces of moisture that could interfere with the derivatization reaction.

Extraction efficiencies for several concentrations of procainamide were determined by adding the internal standard to a known volume of the ethyl acetate after extraction instead of to the serum sample before extraction. Standards representing 100% extraction were prepared by adding both procainamide, at several concentrations, and the internal standard to the ethyl acetate extracts of blank serum samples.

Preparation of Pentafluoropropionyl Derivatives—Derivatization was accomplished by adding 20 μl of pentafluoropropionic anhydride to each residue, tightly capping the tube, and heating at 40° for 25 min. Subsequently, excess derivatizing reagent was removed by evaporation at 40° in a stream of dry air. Evaporation was facilitated by the addition and evaporation of two 100-μl portions of absolute methanol. The derivatized samples were then diluted with 100 μl of ethyl acetate, and 1–3 μl was injected into the gas chromatograph.

Assay of Patient Serum Samples—Aliquots (0.1–1 ml) of 186 serum samples from 16 patients receiving procainamide intravenously were placed in 15-ml siliconized culture tubes containing 0.63 μg of internal standard and were extracted, derivatized, and injected onto the gas chromatograph as described for serum standards. These serum samples were also assayed by the modified colorimetric assay of Sitar *et al.* (9).

GLC—A gas chromatograph[6] equipped with a ^{63}Ni-electron-capture detector was utilized. Glass columns, 1 m long and 4 mm i.d., were packed with 5% OV-17 on 100–120-mesh Gas Chrom Q[7]. The oven temperature was 235° with the injector at 250° and the detector at 350°. Argon–methane (95:5) carrier gas was maintained at a flow rate of 40 ml/min. Prior to attachment to the detector, the packed column was conditioned for 18 hr at 280° with a gas flow of 40 ml/min and was then treated with 3 × 20 μl of silylating agent[8] at 200°.

The pentafluoropropionyl derivatives of procainamide and internal standard had retention times of approximately 5 and 8 min, respectively (Fig. 1).

Calculations—Peak height ratios were calculated by dividing the height of the procainamide peak by the height of the internal standard peak. Standard curves were obtained from data obtained for freshly prepared serum standards and from serum standards kept frozen for up to 3 weeks. The peak height ratios were plotted against the concentration of procainamide base, and the least-squares regression line of best fit was calculated. The concentrations of procainamide in patient samples were derived from the peak height ratios and the regression equation for the standard curve.

The absolute recoveries for several concentrations of procainamide were calculated by dividing the peak height ratio obtained for the serum samples containing a known amount of procainamide, to which the internal standard had been added to the ethyl acetate extract, by the peak height ratio obtained for the serum samples to which both the known amount of procainamide and the internal standard had been added to the ethyl acetate extract.

Mass Spectrometry—A mass spectrometer[9] was used to establish the identity of the pentafluoropropionyl derivatives of both procainamide and the internal standard.

Table I—Standard Curve and Precision Data for Electron-Capture GLC Assay of Procainamide in Human Plasma

Plasma Volume, ml	Procainamide Added, μg/ml	Mean Peak Height Ratio	Procainamide Found, μg/ml
1.0	0.10[a]	0.245	0.101 ± 0.005[b]
	0.30	0.754	0.304 ± 0.005
	0.50	1.23	0.514 ± 0.015
	0.70	1.74	0.720 ± 0.015
	1.00	2.50	1.015 ± 0.023
0.1	1.0	0.244	1.05 ± 0.04
	3.0	0.708	3.09 ± 0.06
	5.0	1.15	5.03 ± 0.10
	7.0	1.60	7.01 ± 0.09
	10.0	2.27	9.96 ± 0.31

[a] $n = 5$. [b] Mean ± *SD*.

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms obtained after derivatization of blank serum and serum containing procainamide and the internal standard. Verification by combined GLC–mass spectrometry confirmed these compounds to be the *N*-pentafluoropropionyl derivatives. Since *N*-acetylprocainamide does not react with pentafluoropropionic anhydride and has very poor intrinsic electron-capturing ability, it is not detectable at usual serum concentrations. In addition, *N*-acetylprocainamide added to serum, 100 μg/ml, and taken through the extraction and derivatization procedures did not give rise to any additional procainamide above that originally present as a contaminant (1.1%). However, at significantly higher derivatization temperatures (>60°) or at incubation times longer than 1 hr, a partial hydrolysis of *N*-acetylprocainamide to procainamide was observed.

Adsorption of procainamide on chromatographic columns and/or

[3] Dr. T. Q. Spitzer, E. R. Squibb and Sons, New Brunswick, N.J.
[4] PCR, Inc., Gainesville, Fla.
[5] Eberbach Corp., Ann Arbor, Mich.
[6] Model 5710A, Hewlett-Packard, Avondale, Pa.
[7] Supelco, Bellefonte, Pa.
[8] Silyl-8, Pierce Chemical Co., Rockford, Ill.
[9] Model 5980A GLC–mass spectrometer equipped with a 5933A data system, Hewlett-Packard, Avondale, Pa.

Table II—Efficiency of Extraction of Procainamide from Human Serum

Plasma Volume, ml	Procainamide Added, μg/ml[a]	Mean Procainamide Found, μg/ml	Mean Recovery, %
1.0	0.10	0.0602	60.2
	0.30	0.188	62.6
	0.50	0.339	67.8
	0.70	0.446	63.7
	1.0	0.683	68.3
0.1	1.0	0.928	92.8
	3.0	2.58	86.0
	5.0	4.26	85.3
	7.0	6.26	89.4
	10.0	9.00	90.0

[a] $n = 4$.

supports was a problem in several previous GLC methods (10–12). With the current procedure, there was no apparent problem with adsorption (Table I); standard curves were reproducible from day to day, provided the chromatographic column was properly conditioned and silanized. It was necessary to replace the first 4–6 cm of the column with fresh packing and to resilanize the entire column monthly.

In preliminary studies, 5% OV-17 proved to be superior to 3 or 10% OV-1 and 3% OV-17. Stationary phases other than 5% OV-17 resulted in significant adsorption and unsymmetrical peak shape for the pentafluoropropionyl derivatives of procainamide and the internal standard.

The proposed procedure has the advantage of requiring a single extraction step instead of the extraction and back-extraction necessary for GLC with flame-ionization detection (13). Preliminary studies indicated that the use of less than 1 ml of phosphate buffer for 0.1-ml serum samples (less than 2 ml for 1-ml serum samples) resulted in the extraction of unacceptable amounts of extraneous material. The amount of sodium chloride was not critical, provided that saturation of the aqueous phase was achieved. It is important that serum standards and not mere aqueous standards be used for calibration curves. The extraction efficiencies of

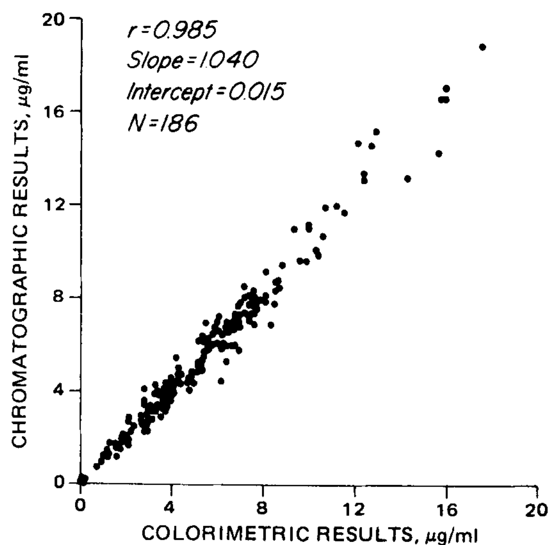

Figure 2—*Graph of procainamide concentration as measured by the electron-capture GLC procedure* versus *that measured by the modified colorimetric procedure (9) in serum samples of patients receiving procainamide.*

the internal standard from serum and water are different (11). There were no differences among calibration curves prepared from human serum from different sources.

Extraction of procainamide from serum resulted in an average absolute recovery of 64.5% for 1-ml samples and of 88.7% for 0.1-ml samples (Table II). Although actual recoveries were quite variable, the inclusion of an internal standard resulted in precise standard curves (Table I).

Comparison of this new GLC technique with the modified colorimetric technique (9) using 186 samples from 16 patients receiving procainamide by long-term intravenous infusion (Fig. 2) yielded a correlation coefficient of 0.985. The results of duplicate GLC analyses of several patient samples gave an average coefficient of variation of 4.5%.

N-Acetylprocainamide has antiarrhythmic activity similar to that of procainamide (12, 15, 16). Therefore, it is important that both procainamide and *N*-acetylprocainamide be measured when evaluating procainamide therapy. The analytical procedure described here would, of course, not be appropriate for that purpose. However, for bioavailability studies requiring several blood samples from the same individual over a relatively short time, the electron-capture GLC technique offers the distinct advantage of requiring very small serum samples. The sensitivity of the method permits the use of very small sample volumes at times of high drug concentration, allowing more blood to be drawn at times of low drug concentration. In addition, the precision of the method is well within the limits necessary for sophisticated pharmacokinetic studies.

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