in mean urine pH with time from the 2nd to the 6th day of antacid administration. The mean pH on the 1st day after discontinuation of the antacid was somewhat above the control value but not statistically significantly so. Urine pH was in the normal control range on the following day.

The results of the experiments to determine the relationship between the dose of the antacid and urine pH are summarized in Table II. Urine pH was determined on the 2nd day of treatment in view of the results obtained in the first part of this investigation. A dose of 7.5 ml four times a day had only a small and not statistically significant effect on urine pH *on the average*.

However, it was noted previously that the magnitude of the antacid effect on urine pH is inversely proportional to the urine pH during the control period (1). The same tendency was apparent in this study; some individuals with low control pH values showed an increase of more than 0.5 pH unit with the 7.5-ml dosing regimen. The 15-ml dosing regimen increased urine pH by more than 0.8 unit on the average, a highly statistically significant difference from the control value. No further increase was observed when the dose of the antacid was raised to 30 ml. Urine pH on the 1st day after discontinuation of the 15- and 30-ml doses was significantly above the control value; this residual effect was particularly pronounced after the 30-ml dose.

The results of this study show that a pharmacokinetically significant increase in urine pH is produced within 24 hr of administration of regular therapeutic doses of a widely used antacid and that this effect persists for about 24 hr after the antacid has been discontinued. The residual effect on urine pH is probably due to the retention of antacid in the GI tract for some time. It is not known if the lack of difference between the effect of the 15- and 30-ml dosage regimens on urine pH is paralleled by a similar lack of dose–response relationship with respect to antacid efficacy.

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GLC Determination of Underivatized Carbamazepine in Whole Blood

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Abstract □ This report describes a rapid GLC assay for carbamazepine in blood. Carbamazepine is chromatographed directly using phenyl methyl silicone gum as the stationary phase. The calibration curve is linear up to 50 μg/ml, with a lower limit of sensitivity of 1 μg/ml. Other anticonvulsants may be measured simultaneously, and the procedure works equally well with plasma or blood.

Keyphrases □ Carbamazepine—GLC analysis, whole blood □ Anticonvulsants—GLC analysis of carbamazepine in whole blood □ GLC—analysis, carbamazepine in whole blood

Carbamazepine is used for the relief of pain from trigeminal neuralgia (1) and in the treatment of epilepsy (2–4). Frequently, patients with complicated seizure disorders are given several other anticonvulsants (*e.g.*, phenytoin, primidone, and phenobarbital) as well as carbamazepine (the usual case in this hospital).

Methods of analysis for carbamazepine that rely on spectrophotometry (5) are complicated by interfering absorbances when mixtures of drugs are analyzed. For such mixtures, GLC is usually the preferred method, and several GLC methods for measuring carbamazepine in serum or plasma recently were reported (6–13). Because of various drawbacks (*e.g.*, tedious extraction requirements, necessity of derivative formation, and inability to use whole blood), none of these methods is considered suitable.

This paper describes a GLC method for the assay for carbamazepine levels in whole blood. This method is simple and rapid and does not require derivative formation. Furthermore, other anticonvulsants can be measured simultaneously.

EXPERIMENTAL

Reagents—Reagent grade chloroform, methanol, and acetic acid were used without further purification. Authentic iminostilbene was obtained commercially[1].

Standards—A stock solution equivalent to 1.0 mg/ml of heptabarbituric acid was prepared by dissolving exactly 108.4 g of dry heptabarbital sodium[2] in 0.9% saline, which had been made alkaline with 5.0 ml of 0.45 *N* sodium hydroxide. The final volume of the solution was adjusted to 100 ml with 0.9% saline. A 20.0-μg/ml working internal standard was prepared by diluting 10.0 ml of the stock solution to 500 ml with 0.9% saline. The 16.3-μg/ml carbamazepine blood standard was prepared by dissolving 4.80 mg of carbamazepine[2] in 10.0 ml of anhydrous methanol and then diluting a 0.34-ml aliquot of this solution with 100 ml of expired blood bank blood. Blood standards of other concentrations were prepared from the carbamazepine stock solution as needed.

Apparatus—The GLC analyses were performed on a gas chromatograph[3] equipped with dual flame-ionization detectors. The chromatograph was fitted with 1.83-m (6-ft) long, U-shaped, glass columns (4.0 mm i.d.) packed with 1.5% phenyl methyl silicone gum (OV-17) on Gas Chrom G HP[4] (80–100 mesh). For the combined analysis of primidone and carbamazepine, a column packed with 1.5% OV-225 on Gas Chrom G HP[4] (80–100 mesh) also was used. The columns were conditioned before use by heating at 290° (260° for OV-225) for 24 hr with 5-ml/min carrier gas flow.

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Ciba Pharmaceutical Co., Summit, N.J.
[3] Varian model 2100.
[4] Applied Science, State College, Pa.

Table I—Effect of Temperature on the Carbamazepine–Iminostilbene Ratio

Initial Column Temperature[a]	Peak Height Ratio[b], Carbamazepine to Iminostilbene
180°	1.02
	1.25
	1.48
190°	0.96
	1.24
	2.46
200°	0.82
	2.10
	3.60
210°	1.03
	3.43
	1.67
220°	1.39
	1.03
	1.40
230°	2.66
	1.20
	1.48
240°	3.24
	1.06
	1.80
250°	1.62
	1.65
	1.23
260°	2.50
	0.97
	1.91

[a] Column temperature was programmed to 266° at 6°/min. [b] Measurements were made from chromatograms of extracts of a 10.0-μg/ml carbamazepine standard.

The chromatography settings for the OV-17 column were as follows: nitrogen as carrier gas, 30 ml/min; air, 300 ml/min; hydrogen, 30 ml/min; injection port and detector temperatures, 270°; initial column temperatures, 180°; and program rate, 6°/min up to 266° followed by isothermal heating at 266° for 10 min. For the OV-225 column, the initial column temperature was 230° and the program rate was 10°/min up to 270°.

The mass spectral analysis was performed on a gas chromatograph–mass spectrometer[5] under the following conditions: electron energy, 60 ev; emission current, 450 μamp; electron multiplier[6], 2.2 kv; preamplifier range, 10^{-7} amp-v^{-1}; and GLC conditions, same as for OV-17 column.

Extraction Procedure—To a 25-ml glass vial were added 2.0 ml of whole blood (ethylenediaminetetraacetic acid as anticoagulant), 1.0 ml of the 20.0-μg/ml heptabarbital working standard, and 10 drops of acetic acid. The vial was swirled gently for a few seconds, and 10 ml of chloroform was added. After being capped tightly, the vial was shaken vigorously for 5 min mechanically.

The solution was allowed to settle for 30 sec, and the chloroform layer was separated (the congealed blood cells usually adhered to the sides of the vial) and evaporated to dryness under a gentle stream of air in a 60° water bath. (For plasma, the solution was first filtered through 1 g of anhydrous sodium sulfate.) The residue was reconstituted with 50 μl of chloroform, and 5 μl of this solution was injected into the chromatograph.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram of an extract from 2 ml of blood containing 16.3 μg/ml of carbamazepine to which 20 μg of heptabarbital had been added. As previously reported (10–13), a substantial percentage of the carbamazepine had decomposed to a second compound with a relative retention time of 0.85. This peak also was found when a pure sample of carbamazepine was chromatographed directly.

It has been reported (11, 13) that carbamazepine undergoes on-column decomposition to give iminostilbene; under the given GLC conditions, an authentic sample of iminostilbene had the same relative retention time as this peak (0.85 relative to heptabarbital). Likewise, the mass spectra of authentic iminostilbene and the decomposition product were identical (base peak and M^+ both at *m/e* 193). From these findings it was concluded that the decomposition product was iminostilbene.

Table I shows that this transformation was not strictly a function of temperature. That is, the ratio of carbamazepine to iminostilbene was variable at all temperatures studied. Even with derivatization (10, 13), the problem of iminostilbene formation still exists, although the authors claim that accurate quantitation of carbamazepine can still be achieved. In view of these results, it was felt that any GLC assay for carbamazepine should also account for the formation of iminostilbene.

[5] Finnigan model 1015D.
[6] Bendix.

Table II—Replicate Chromatograms of a 16.3-μg/ml Carbamazepine Blood Standard

Run	Relative Response[a]		
	Iminostilbene	Carbamazepine	Total
1	0.68	0.96	1.64
2	0.87	0.79	1.66
3	0.70	0.96	1.66
4	0.81	0.86	1.67
5	0.43	1.20	1.63
6	0.44	1.22	1.66
7	0.63	0.96	1.59
8	0.87	0.82	1.69
9	0.53	1.17	1.70
10	0.39	1.24	1.63
11	0.54	1.12	1.66
12	0.51	1.14	1.65
13	0.56	1.08	1.64
14	0.52	1.12	1.64
15	0.54	1.03	1.57
16	0.45	1.24	1.69
17	0.64	1.06	1.70
18	0.54	1.17	1.71
19	0.53	1.15	1.68
20	0.59	1.14	1.73
		Mean =	1.66
		SD =	±0.04
		CV =	2.3%

[a] Relative response = height of peak/height of internal standard. Concentration of internal standard = 10.0 μg/ml.

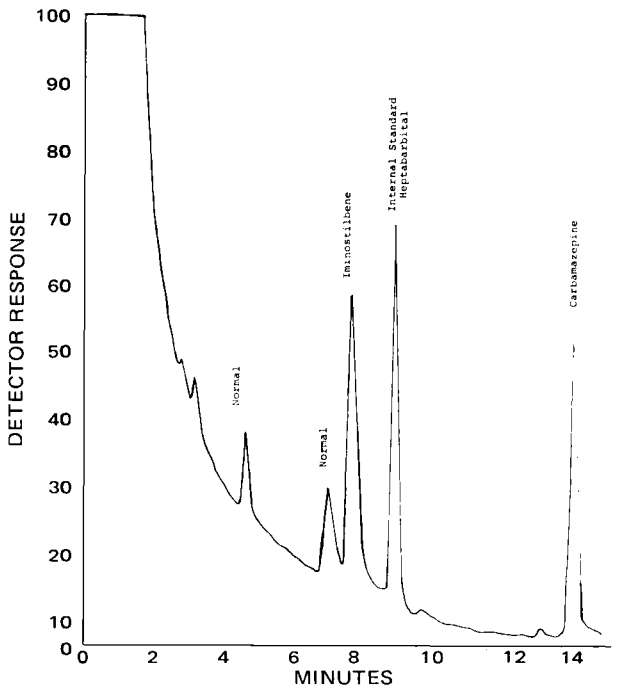

Figure 1—*Chromatogram of extract from 2 ml of blood containing 3.3 μg of carbamazepine.*

Table III—Recovery Study

Amount of Drug Added to Blood Pool, μg/ml	Amount of Drug Recovered, μg/ml	Relative Recovery, %
2	1.8	90
5	5.7	114
10	9.3	93
20	18.0	90
40	40.5	101
80	86.0	107
100	101.0	101

Table IV—Relative Retention Times of Various Anticonvulsants

Drug or Metabolite	OV-17[a]	OV-225[a]
Ethosuximide	0.08	—
Methsuximide (as metabolite)	0.50	0.46
Iminostilbene	0.85	0.70
Phenylethylmalonamide	0.86	0.93
Phenobarbital	0.89	—
Heptabarbital (internal standard)	1.00	1.00
Primidone	1.58	—
Carbamazepine	1.63	1.83
Phenytoin	1.68	2.41

[a] See *Experimental* for GLC conditions.

It seemed likely that whereas the ratio of carbamazepine to iminostilbene might vary from one run to the next for any given concentration of carbamazepine, the sum of the relative responses of these two drugs might not vary; and if the sum of the relative responses did not vary, then carbamazepine could be quantitated by simply comparing the sum of the areas (peak heights) of the two peaks to that of an internal standard. This assumption proved to be correct: the ratio of carbamazepine to iminostilbene varied from 2.5 to 0.9 for 20 consecutive runs of a 16.3-μg/ml blood standard whereas the sum of the relative responses for this standard did not vary by more than 2.5% from the mean (Table II). Thus, the carbamazepine concentration was linearly related to the sum of the relative responses of carbamazepine and iminostilbene over the range investigated (up to 50 μg/ml). Recovery of carbamazepine from whole blood ranged from 90 to 114% (Table III), and the between-run standard deviation was only 0.4 μg/ml, with a lower limit of sensitivity of approximately 1 μg/ml.

The other anticonvulsants that can be determined simultaneously with this method are listed in Table IV. For the combination of primidone and carbamazepine, the phenylethylmalonamide metabolite is not completely resolved from iminostilbene on the OV-17 column. For a patient on these drugs, it is necessary to estimate and subtract the amount of phenylethylmalonamide in the combined peak by taking advantage of the fact that phenylethylmalonamide and iminostilbene are completely resolved on an OV-225 column (see *Experimental* for GLC conditions). The blood extract is chromatographed on both OV-17 and OV-225 columns, and the amount of phenylethylmalonamide (relative to the standard) is estimated from the latter chromatogram (the ratio of phenylethylmalonamide to internal standard did not vary from one run to another).

Various therapeutic ranges for carbamazepine in serum or plasma have been reported (3–8), with consensus being a range of 4–13 μg/ml. Since this assay used whole blood, it was necessary to compare blood with plasma or serum levels of carbamazepine to determine whether therapeutic ranges could be correlated. This precaution was felt to be necsssary due to the well-known (14) dilutional effect of red cells on the concentration of some drugs. Accordingly, whole blood specimens from several patients were obtained, and one portion of each was analyzed as blood and the other portion was spun down and analyzed as plasma. *No* significant differences for carbamazepine levels were observed between the two different specimens for these patients.

To determine the linearity of this comparison, whole blood standards of carbamazepine were prepared and analyzed in the same manner, and again no appreciable differences were found up to the highest concentrations studied (50 μg/ml). In addition, this comparison shows that either plasma or whole blood can be used in this assay. From the limited analyses carried out to date (less than 10 patients), a therapeutic range has not been determined. Levels between 2 and 8 μg/ml have been found, but other anticonvulsants as well as carbamazepine were being administered to these patients. Further clinical studies are necessary to determine the therapeutic range or ranges of carbamazepine in combination with various other anticonvulsants.

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