Measurement of Antidiabetic Sulfonylureas in Serum by Gas Chromatography with Electron-capture Detection

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SUMMARY

A method is described for measurement of chlorpropamide, tolbutamide, and the tolbutamide metabolites hydroxymethyltolbutamide and carboxytolbutamide in blood. It consists of formation of the thermally stable methyl-trifluoroacetyl derivatives of chlorpropamide and tolbutamide, and the methyl-heptafluorobutyryl derivatives of hydroxymethyltolbutamide and carboxytolbutamide, which are then analyzed by electron-capture gas chromatography. Measurements of blood levels of the compounds with this method have been verified by quantitation of the same samples using gas chromatography-mass spectrometry in the mass fragmentography mode. Blood level values and β-phase half-time disappearance rates of chlorpropamide, tolbutamide, hydroxymethyltolbutamide, and carboxytolbutamide were measured in normal volunteers following an oral dose of chlorpropamide or tolbutamide. Blood levels of the four compounds were also determined in a few diabetics receiving continuous daily treatment. DIABETES 26:50-57, January, 1977.

Although oral antidiabetic sulfonylureas have been used in the treatment of nonketotic maturity-onset diabetes mellitus for 20 years, much remains to be learned about their pharmacology. Their mechanism of action in lowering blood sugar is still not understood. The possibility that at least one of them, tolbutamide, may have deleterious effects on the cardiovascular system has been the subject of heated controversy in the public press as well as the current medical literature.[1-5]

One reason for such lack of information is the unavailability or the imprecision of techniques for the quantitation of sulfonylureas and their metabolites in blood. The recent development in our laboratory of a method for the preparation of methyl-perfluoroacyl derivatives of sulfonylureas (figure 1) that are stable at the high temperatures used in gas chromatography permits the measurement of tolbutamide (TB)* and chlorpropamide (CP) by gas-liquid chromatography with flame ionization detection.[6] Because methylperfluoroacyl derivatives have electron-capture properties it has been possible to devise a more sensitive technique employing gas chromatography with electron-capture detection (EC-GLC). This more sensitive technique is necessary for measurement of drug metabolites that are present in blood at low concentrations. The method is described in detail in this paper. Data on the blood levels of CP and TB in diabetics and the disappearance of CP, TB, and the two major metabolites of TB from blood have been obtained by EC-GLC and verified by analysis of the same samples with the specific mass spectrometric method of mass fragmentography.

*The following nonstandard names and abbreviations are used: TB, tolbutamide; hydroxymethyltolbutamide, OH-TB, 1-butyl-3-(4-hydroxymethylbenzenesulfonyl) urea; carboxytolbutamide, COOH-TB, 1-butyl-3-(4-carboxybenzenesulfonyl) urea; CP, chlorpropamide; pCBSU, *p*-chlorobenzenesulfonylurea; pCBSA, *p*-chlorobenzenesulfonamide; 2-OH-CP, 1-[(*p*-chlorophenyl)sulfonyl]-3-(2-hydroxypropyl)-urea; 3-OH-CP, 1-[(*p*-chlorophenyl)sulfonyl]-3-(3-hydroxypropyl)-urea; Me, methyl; TFA, trifluoroacetate; PFP, pentafluoropropionate; HFB, heptafluorobutyrate; EC-GLC, gas chromatography with electron-capture detection; GC-MS, gas chromatography-mass spectrometry.
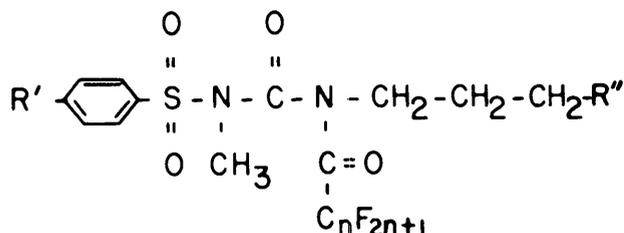
$$R' - \langle \bigcirc \rangle - \overset{O}{\underset{O}{\overset{\|}{S}}} - \overset{}{\underset{CH_3}{N}} - \overset{O}{\overset{\|}{C}} - \overset{}{\underset{\underset{C_nF_{2n+1}}{C=O}}{N}} - CH_2-CH_2-CH_2-R''$$

FIG. 1. General structure of sulfonylurea methyl-perfluoroacyl derivatives. Tolbutamide: R' = R'' = CH₃; chlorpropamide: R' = Cl, R'' = H; hydroxymethyltolbutamide: R' = CnF2n + 1COOCH2; R'' = CH3; carboxytolbutamide: R' = CH3OOC, R'' = CH3; TFA: n = 1; PFP: n = 2; HFB: n = 3.

MATERIALS AND METHODS

Reagents and chemicals. Tolbutamide (TB) and chlorpropamide (CP) reference compounds were donated by Dr. Francis Henderson, of the Upjohn Company. Hydroxymethyltolbutamide (OH-TB), carboxytolbutamide (COOH-TB), and diazomethane were prepared as described previously.[7] The CP metabolites *p*-chlorobenzenesulfonylurea (pCBSU), *p*-chlorobenzenesulfonamide (pCBSA), 1-[(*p*-chlorophenyl)-sulfonyl]-3-(2-hydroxypropyl)-urea (2-OH-CP), and 1-[(*p*-chlorophenyl)sulfonyl]-3-(3-hydroxypropyl)-urea (3-OH-CP) were supplied by Pfizer, Inc. Nanograde solvents (Mallinckrodt) were used as supplied. Trifluoroacetic anhydride and heptafluorobutyric anhydride were obtained from Pierce Chemical Co. All glassware was washed in Contrad-70 (Scientific Products) and rinsed with 0.1 N HCl before drying.

Extraction of blood samples. To determine the recovery of sulfonylureas from blood, stock solutions in methanol were added separately to 15-ml. conical centrifuge tubes in amounts to give final serum concentrations of 1.38, 2.76, 5.52, 11.04, 22.08, and 44.16 μg./ml. of CP; 2.7, 5.4, 10.8, 16.02, and 21.6 μg./ml. of TB; and 0.5, 1, 2, and 4 μg./ml. of OH-TB and COOH-TB. After the methanol was removed with a stream of N₂, the sulfonylureas were gently mixed with normal human serum to give the appropriate concentration. For measurement of CP and TB, 50-μl. aliquots of the spiked serum samples were acidified with an equal volume of 1 N HCl and extracted by vortexing for one minute with 5 ml. of ethyl acetate saturated with water. The solvent layers were separated by centrifugation; 1 ml. of the ethyl acetate layer was transferred to a 15-ml. conical centrifuge tube with a Teflon-lined screw cap and dried

with N₂. For measurement of OH-TB and COOH-TB, 500 μl. of serum was extracted twice with 5 ml. of ethyl acetate, and 2 ml. of the combined fraction was taken.

To prepare reference curves, the same amounts of sulfonylurea were placed in conical tubes and dried with N₂. Human serum (0.05 ml.) from a normal subject acidified with an equal volume of 1 N HCl was extracted with a 100-fold excess of ethyl acetate (water-saturated). One milliliter of the ethyl acetate layer was added to each of the tubes containing the sulfonylureas and dried with N₂.

Formation of methyl-perfluoroacyl derivatives. The ethyl acetate extracts were dissolved in 50 μl. of methanol and reacted five minutes at room temperature with 0.5 ml. of ethereal diazomethane. The solution was then evaporated with N₂. Perfluoroacyl derivatives were formed by dissolving the methylated sulfonylureas in 100 μl. of ethyl acetate:pyridine (10:1), adding 20 μl. of trifluoroacetic anhydride (heptafluorobutyric anhydride for determination of OH-TB and COOH-TB), and incubating in the capped tubes for 30 minutes at 70°. The mixture was allowed to cool and then dried with N₂. Five milliliters of cyclohexane was added to each tube along with approximately 1 gm. of anhydrous sodium sulfate and mixed on a vortex mixer. The derivatives were stable in this form for several days at 4°.

Gas chromatography. Samples were analyzed on a Varian 2100 Gas Chromatograph equipped with a 63Ni electron-capture detector. The injector temperature was 230°, and the detector temperature was 275°. Derived TB and CP were chromatographed isothermally at 185° on a 1.8 m. × 2 mm. I.D. glass column of 3 per cent OV-1 on 80/100 Supelcoport, N₂ carrier gas 30 ml./min. Tolbutamide metabolite derivatives were chromatographed isothermally at 205° on 1.8 m. × 4 mm. I.D. glass columns of 3 per cent OV-17 on 100/120 Gas Chrom-Q, N₂ carrier gas 45 ml./min. Quantitation was achieved by measurement of the chromatographic peak height relative to that of a reference compound injected simultaneously and by comparison with a standard curve prepared the same day. Tolbutamide-methyl-trifluoroacetate (Me-TFA) was used as the reference compound for CP measurement and CP-Me-TFA the reference for TB measurement. Hydroxymethyltolbutamide-Me-TFA was used as the external standard for measurement of OH-TB and COOH-TB as the methyl-heptafluorobutyryl (Me-HFB) derivatives. The over-all procedure for measurement of sulfonylureas in blood is outlined in diagram 1.

Mass fragmentography. Gas chromatography-mass spectrometry (GC-MS) using the mass fragmentography technique[8] was carried out on a Finnigan 1015D GC-MS interfaced with a Systems Industries System 150 data-acquisition and control system. Ion energy was 70 eV. For mass fragmentography of chlorpropamide as the Me-TFA derivative, ions m/e 153, 175, and 195 of the CP-Me-TFA spectrum and m/e 153 of the TB-Me-TFA spectrum were recorded at 400 msec. each. The compounds were chromatographed at 190° on a 1 m. × 2 mm. I.D. column of 3 per cent OV-1, with injector and separator temperatures 240°.

For mass fragmentography of tolbutamide-methyl-pentafluoropropionate (TB-Me-PFP), ions m/e 203 and 259 of the TB-Me-PFP spectrum were recorded at 100 and 500 msec., respectively. The 203 and 245 of the external standard CP-Me-PFP recorded at 100 and 500 msec, respectively. The compounds were chromatographed on a 1.5 m. × 2 mm. I.D. glass column of 3 per cent OV-17.

Mass fragmentography of the tolbutamide metabolites was performed on ions m/e 199 of COOH-TB-Me-HFB, m/e 303 of OH-TB-Me-HFB, and m/e 209 of the external standard OH-TB-Me-TFA. Ions were scanned 200 msec. each and signals for three scans averaged. Compounds were chromatographed on a 1.22 m. × 2. mm. I.D. glass column of 1 per cent

DIAGRAM 1

Determination of tolbutamide and chlorpropamide in serum*

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50 μl. serum
    |  50 μl. 1 N HCl
    |  5 ml. ethyl acetate
  ┌─────────┴─────────┐
aqueous          ethyl acetate (1 ml.)
                    |  dry with N₂
                    |  react with CH₂N₂ at room temp. for 5 min.
                    |  dry with N₂
                    |
                 N-methyl sulfonylureas
                    |  dissolve in 110 μl. of 10% pyridine
                    |    in ethyl acetate
                    |  react wtih 20 μl. trifluoroacetic
                    |    anhydride at 70° for 30 min.
                    |  dry with N₂
                    |
                 Sulfonylurea-Me-TFA derivatives
                    |  dissolve in 5 ml. cyclohexane
                    |    containing standard
                    |
                 Inject 1 μl. onto GLC with
                    electron-capture detector
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*To determine OH-TB and COOH-TB, 500 μl. of serum is extracted 2× with ethyl acetate, and 2 ml. of the organic phase is taken for formation of Me-HFB derivatives.

OV-17, at 200°.

Blood level data. Studies with normal volunteers and diabetics on treatment were carried out after informed consent was obtained. Samples from a patient with CP-induced hypoglycemia were obtained during the course of routine medical care. Quantitative measurements were made by extrapolation of relative peak height values from a standard curve prepared the same day. Blood level values were plotted semilogarithmically vs. time, and β-phase half-time disappearance rates (t½βs) calculated from the graph.

RESULTS

Gas chromatography of serum sulfonylureas. The thermally stable methyl-perfluoroacyl derivatives of the sulfonylureas afforded a sensitive and relatively specific electron-capture gas chromatographic method of measurement of these compounds in serum extracts. The sensitivity to electron-capture detection was high: 2.8×10^{-16} moles/sec. for TB-Me-TFA and 2.6×10^{-16} moles/sec. for CP-Me-TFA.

Figure 2 indicates that the serum blank was clean in the region where the CP and TB derivatives eluted even though a simple one-step extraction was used. The concentration of the TB metabolites in blood is approximately one tenth that of the parent compound, and a larger fraction therefore had to be injected on the chromatograph in order to quantitate these compounds. By chromatographing on OV-17 as the Me-HFB derivatives, OH-TB and COOH-TB eluted in noise-free regions of the chromatogram, and more extensive cleanup procedures were not necessary.

Extraction of sulfonylureas from serum. The efficiency of the method for extraction of blood samples was examined by comparison of curves of the peak height ratios of compounds prepared from spiked blood with standard curves of nonextracted compounds prepared simultaneously. Figure 3 contains the curves obtained from extraction of CP, TB, and the TB metabolites, respectively. It can be seen that good linearity of the peak height ratio response was obtained with all compounds and that the recoveries from serum were quantitative over the range of blood levels expected in patients receiving the drugs. Therefore it was unnecessary to add an internal standard to serum before extraction, and the use of an external standard for gas chromatography was possible. We have found that during the preparation of standard curves it is important to add an ethyl acetate extract of blank serum to tubes containing the known quantities of drug; there will otherwise be significant adsorption of the sul-
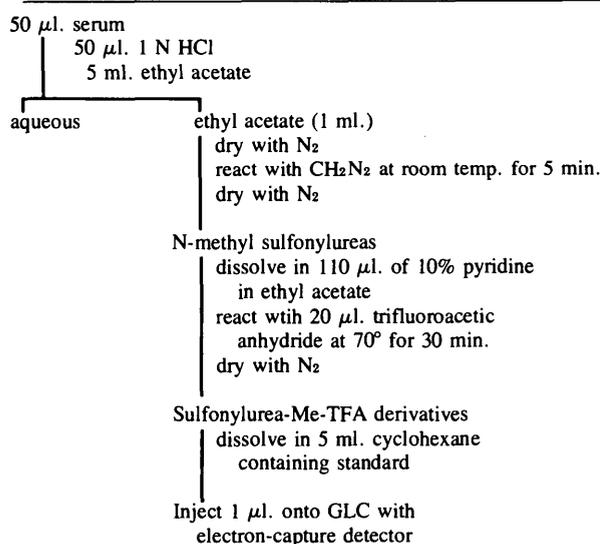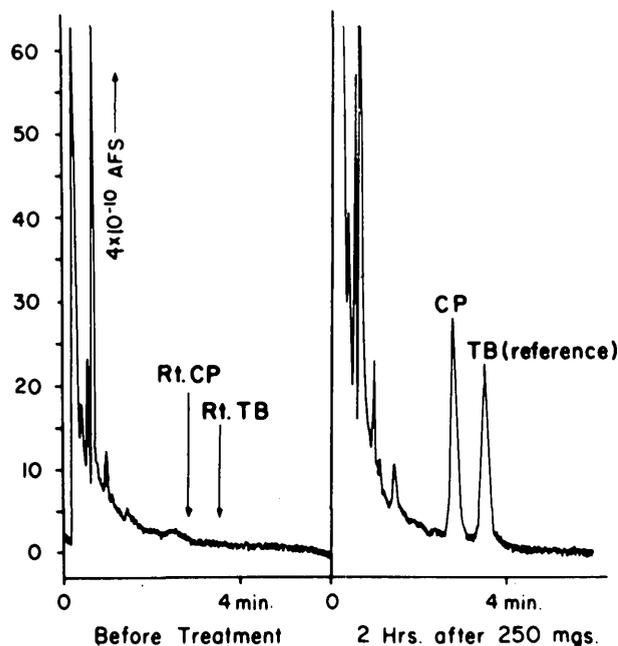
FIG. 2. EC-GLC of CP in the serum of a volunteer two hours after oral ingestion of 250 mg. CP was chromatographed as the Me-TFA derivatives on a 1.8 m. × 2 mm. column of 3 per cent OV-1 at 185°. The external standard was tolbutamide-Me-TFA. A serum sample obtained before treatment is shown for comparison, and the retention times (Rt) of each drug are indicated to illustrate the low background.

fonylurea onto glass. In addition, it is helpful to rinse the glassware with weak mineral acid, as described in the Methods section.

The TB metabolites were measured as the Me-HFB derivatives. Me-PFP derivatives also chromatograph well, but we have encountered problems in obtaining reagent pentafluoropropionic anhydride that is free from small amounts of trifluoroacetic anhydride. Because the Me-TFA derivatives of TB migrate very close to the Me-PFP derivative of the TB metabolite, OH-TB,[7] erroneous results could be obtained as the

result of contamination with TB-Me-TFA. Heptafluorobutyric anhydride is free from contamination with other perfluoroacid anhydrides and is therefore a more suitable choice for the determination of TB metabolites.

The methyl-perfluoroacyl derivatives of CP, TB, and COOH-TB are stable to hydrolysis for several days at room temperature if kept in cyclohexane over anhydrous sodium sulfate. However, the OH-TB derivatives do not keep as well and should be analyzed the day they are derived.

Confirmation of EC-GLC blood level measurements by mass fragmentography. The highly specific GC-MS method of mass fragmentography was also used to quantitate CP in the same derived samples analyzed by EC-GLC. Since mass fragmentography is somewhat less sensitive than EC-GLC, derived samples were concentrated 10-fold. Figure 4 is a mass fragmentogram of a serum sample obtained 28.5 hours after an oral dose of 250 mg. CP. The specific ions at m/e 153, 175, and 195 identify the CP-Me-TFA at the correct relative retention time, and the ion m/e 153 also indicates the reference standard TB-Me-TFA. The origins of these ions in the mass spectra of TB and CP methyl-perfluoroacyl derivatives have been discussed in an earlier paper.[7] Quantitation by mass fragmentography was also achieved by peak height ratios and extrapolation from standard curves. The levels of CP determined by both EC-GLC and mass fragmentography in blood samples of a normal volunteer after ingestion of 250 mg. CP and in samples of a patient with drug-induced hypoglycemia are compared in figure 5. Close agreement between the two methods is seen on measurement of CP levels from both subjects.
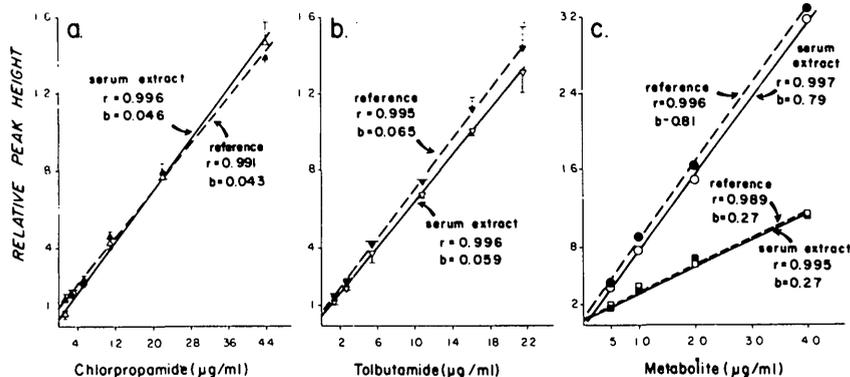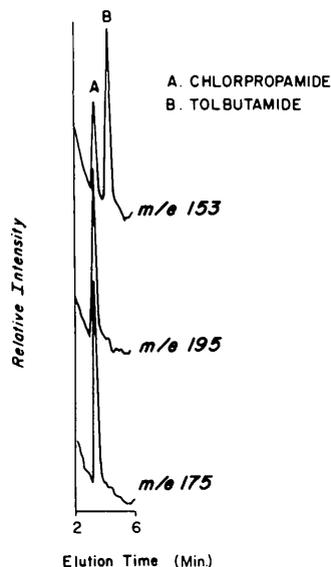
FIGURE 3

(A) Recovery of chlorpropamide from blank serum to which the drug was added (△—△) compared with the reference curve (▲---▲), as determined by EC-GLC. Peak heights were measured relative to the TB-Me-TFA standard (0.2 pmoles). The mean (n=3) and standard deviation are given for each point, r = correlation coefficient, b = slope. (B) Recovery of tolbutamide from blank serum to which the drug was added (▽—▽) compared with the reference curve (▼---▼). Peak heights were measured relative to the CP-Me-TFA standard (0.2 pmoles). (C) Recovery of OH-TB (O—O) and COOH-TB (□—□) from blank serum to which the two TB metabolites were added compared with respective reference curves (●---●) and (■---■). Peak heights were measured relative to the OH-TB-Me-TFA standard (0.4 pmoles).

FIGURE 4

Mass fragmentography of serum chlorpropamide 28.5 hours after oral ingestion of 250 mg. by a normal individual. Ions m/e 153, 175, and 195 of CP-Me-TFA (A.) and 153 of the external standard TB-Me-TFA (B.) were scanned at 400 msec. each (ion energy 70 eV) The column was 1 m. × 2 mm. I.D., packed with 3 per cent OV-1, and chromatographed at 190°.

A plot of EC-GLC vs. mass fragmentography values of CP is shown in figure 6. The blood levels of TB and metabolites in a patient treated with 500 mg. of TB were determined by EC-GLC and mass fragmentography and are also shown in figure 6. The two methods of measurement were in close agreement, as shown by the high correlation coefficients (r), and slopes (b) close to 1. The hydroxymethyltolbutamide-Me-HFB derivative is more susceptible than the others to hydrolysis in solution with time (see above), and the lower slope of this compound in figure 6 reflects some loss of derivative between the time of EC analysis and mass fragmentography. The close agreement of the results obtained by the two procedures thus validates the simpler EC-GLC method for future routine work.
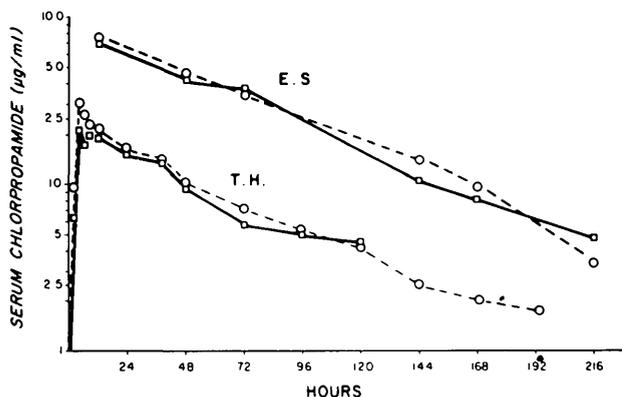

FIG. 5. Serum CP levels in a normal volunteer, T.H., after 250 mg. p.o. at time 0, and a patient, E.S., who developed chlorpropamide-induced hypoglycemia after having been on 250 mg. p.o. b.i.d. Values determined by mass fragmentography (□ —□) and EC-GLC(O---O) correlate very well.

Measurement of blood levels during treatment with CP and TB. After four normal volunteers received an oral dose of 250 mg. CP, 2-ml. blood samples were taken at frequent intervals for several days. Following 250 mg., maximum CP values of 23-31 μg./ml. were attained in four to six hours. The half-time disappearances from blood in the four patients were 39, 46, 56, and 57 hours. Figure 7 shows what happened to blood levels of CP following daily administration to three diabetics who were started on drug therapy. Serum CP increased in a stepwise fashion for a period of several days until plateau levels of 40-80 μg./ml. were attained on 250 mg. a day (patients LL and MS) and 170 μg./ml. on the 250-mg. b.i.d. dose (patient EB).
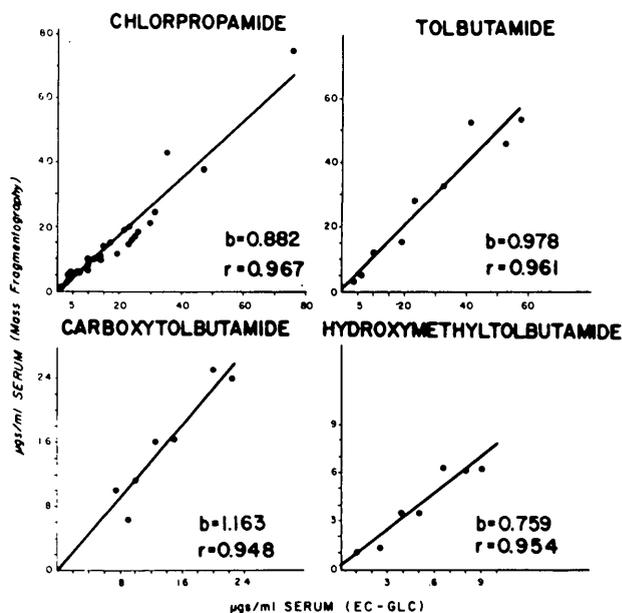

FIG. 6. Correlation of sulfonylurea blood levels values from treated patients as determined by EC-GLC (abscissa) and mass fragmentography (ordinate). The correlation coefficient (r), the slope (b), and the solid line plotted were obtained by least-squares regression analysis.

The disappearance of TB from the blood was determined in four normal volunteers following a single dose of 500 mg. Maximum values of 54-64 μg./ml. were reached in two to three hours in three subjects, while a fourth showed slower absorption, reaching a maximum serum concentration of 33 μg./ml. in six hours. The half-time disappearance from blood ranged from 5½ to 13 hours in these subjects. OH-TB and COOH-TB disappeared from blood at rates similar to or faster than that of the parent drug (figure 8).

The serum levels of TB and metabolites following consecutive daily doses were also measured in a patient
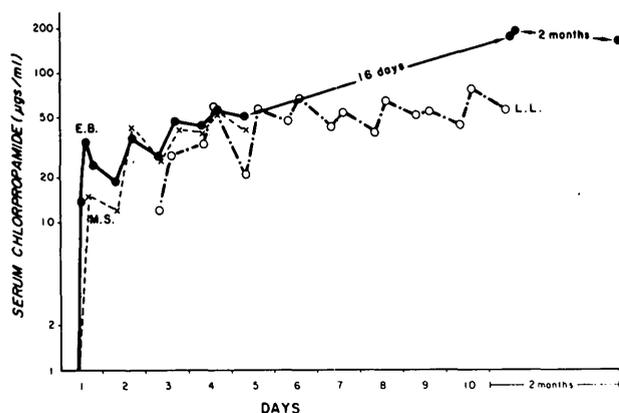
FIG. 7. The staircase effect of consecutive daily treatment on blood levels of CP. Samples were obtained immediately before and three to four hours after daily administration of CP. Patient EB (●—●) received 250 mg. q.d. to 4½ days, then 250 mg. b.i.d.; patient MS (X---X) and LL (O----O) received 250 mg. q.d. Quantitation was by the EC-GLC method.

beginning treatment with TB. Blood samples were taken prior to and four to six hours after a daily dose of 500 mg. of TB. Figure 9 shows that no staircase effect was evident. Levels of serum TB, COOH-TB, and OH-TB stayed at essentially the same levels observed in the normal volunteers (figure 8).

DISCUSSION

Lack of adequate methodology for measurement of blood levels of the sulfonylurea drugs and metabolites has hindered studies of the pharmacokinetics, blood level-response relationship, and mechanism of action of these compounds in vivo. Several approaches to quantitative analysis of blood levels of this class of drug have been attempted in the past. Spectrophotometric and colorimetric procedures not only lack sensitivity but are susceptible to interference by other drugs[9] or their own metabolites[10] unless complicated solvent partition procedures are employed.[11] High-pressure liquid chromatography has been suitable for analysis of pharmaceutical preparations[12] or urinary fractions[13] but lacks the sensitivity for measurement of blood levels. Gas-chromatographic procedures have heretofore been hindered by the thermal lability of sulfonylureas. Because stabilization of the drugs by formation of N-methyl derivatives[14-16] has not always been reproducible,[12,17] several laboratories have attempted measurement of tolazamide and tolbutamide by quantitative thermal degradation to N-methyl-p-toluenesulfonamide,[17,18] but this approach does not allow measurement of metabolites.

Other methods, such as chemical ionization mass spectrometry employing stable isotope dilution,[19] although accurate and sensitive, required specialized equipment not generally available to clinical laboratories and are thus suitable only for very limited studies of the compounds.

Methyl-perfluoroacyl derivatives of sulfonylureas provide a number of advantages for measurement. They impart the required thermal-stability and vapor-phase properties[7] for GLC analysis, with its inherently great sensitivity and specificity. They impart additional electron-capturing properties to the molecules and thus further increase sensitivity and specificity. They are quickly and easily prepared so that measurements by GLC analysis can be semiautomated.

Although mass fragmentography is becoming widely used as a quantitative method for measurement of a number of compounds in biomedicine, it requires expensive specialized equipment not generally available at the present time. Therefore, in the present study, mass fragmentography was used only to validate the simpler, more generally applicable procedure of EC-GLC. Since the mass fragmentography was done on samples obtained from actual patients or volunteers treated with the drug and not on blank serum to which sulfonylureas were added, there is assurance against interference from unknown drug metabolites. We have not obtained any information to date as to whether other drugs administered to patients concurrently will be a problem. To cause interference, such drugs or their metabolites would have to migrate at the same retention time and also possess electron-capture properties.
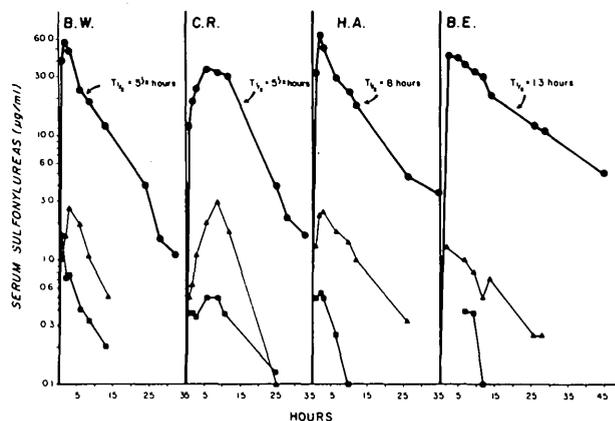

FIG. 8. Blood levels of TB (●—●), COOH-TB (▲—▲), and OH-TB(■—■) in four normal volunteers following ingestion of 500 mg. Determinations were by the EC-GLC method.

The blood levels of CP found four to six hours after a single dose of 250 mg. Diabinese (Pfizer) (23-31 μg./ml.) are similar to those obtained in CP bioavailability studies performed by Monroe and Welling[20] with a TLC-fluorometric assay. The CP elimination phase half-times (39-57 hours) are also in agreement with literature values obtained by a variety of procedures.[10,13,21] Brotherton et al.[10] showed that the half-time measurements obtained by older methods could be influenced by the metabolites pCBSA and pCBSU, although their method did not account for the 2-OH-CP and the 3-OH-CP metabolites. Preliminary data indicate that the four known metabolites of CP do not interfere with the measurement of the parent compound. Methyl-trifluoroacetyl derivatives of 2-OH-CP and 3-OH-CP chromatograph on 3 per cent OV-1 with relative retention times to CP of 1.26 and 1.82, respectively. The pCBSA and pCBSU did not form derivatives that give visible peaks when chromatographed under these conditions.

The need for a method specific for unchanged chlorpropamide is evident from the confusion in the literature regarding the build-up and blood clearance of this compound during chronic treatment, especially regarding instances of chlorpropamide-induced hypoglycemia.[22,23] Although some workers have attempted to correct for pCBSU levels, none have eliminated the possibility of concurrent measurement of 2-OH-CP, which is a major CP metabolite (55 per cent) in urine.[13] Nothing as yet is known of the effect of long-term treatment on blood levels of this metabolite or of its significance in drug-induced hypoglycemia.

The serum half-times of the disappearance of tolbutamide in four normal volunteers given 500 mg. of Orinase (Upjohn) orally (figure 8) were similar to values in the range of 4-11 hours obtained by other procedures[9,24-29] after a variety of doses and routes of administration. The major tolbutamide metabolite in the serum of the four individuals in the present study was COOH-TB (figure 8), confirming results obtained following 1 gm. i.v. on a single subject by Matin and Knight,[19] who used a stable-isotope dilution procedure. The metabolite OH-TB was detected in blood for only a short period of time following an oral dose of 500 mg. TB (figure 8).

The constancy of blood levels of tolbutamide and metabolites after consecutive daily administration of 500 mg. TB to one patient with mild diabetes (figure 9) is not surprising, given the short half-time disappearance of these compounds. Throughout four
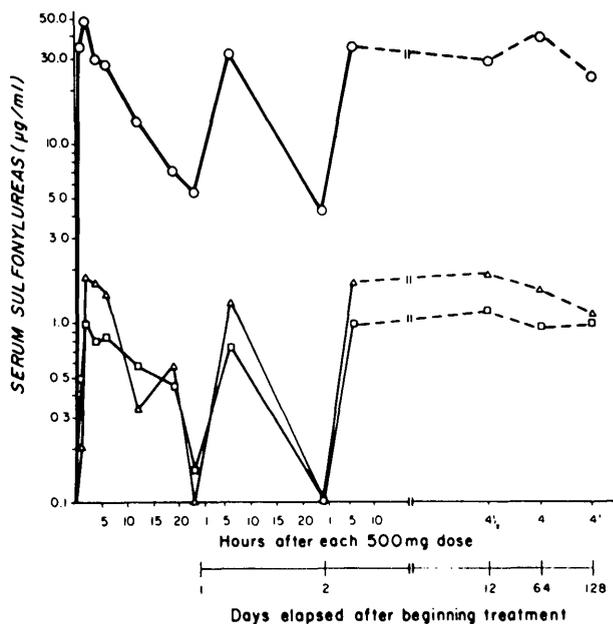

FIG. 9. Blood levels of TB (O—O), COOH-TB (Δ—Δ), and OH-TB (□—□) determined by EC-GLC following consecutive daily doses in a diabetic patient beginning treatment with 500 mg. TB daily.

months of treatment there was no detectable change in the relative proportions of the parent drug and metabolites, indicating that the mode of metabolism did not change. Other groups of investigators have already suggested that the rate of tolbutamide metabolism is not increased in man during long-term treatment,[30,31] but there is at least one report of some reduction in the plasma half-time during the first two to five months of treatment.[24] The effect of chronic treatment on blood levels of the tolbutamide metabolites has not to our knowledge been studied previously.

Consecutive daily treatment with CP, on the other hand, resulted in a stepwise build-up of this compound in blood for several days until plateau levels were reached (figure 7), results comparable to an earlier report by Carlozzi et al.[32]

The examples given here of studies with volunteers and patients are intended to illustrate the ease and versatility of the EC-GLC method for measurement of a number of sulfonylureas and metabolites in blood. Tolazamide can also be determined by this method.[7] Much remains to be learned, by procedures such as we describe, concerning the accumulation of these drugs and their metabolites in blood in a variety of clinical conditions, particularly in patients with compromised liver and kidney function.

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