the connectivity indexes, influence the degree of potency as manifested in this test.

DISCUSSION

The set contains three subclasses of nitrosamines: alkyl, guanidino, and ureido derivatives. Within the alkyl subset are three cyclic derivatives. The equations treat these subsets equally well for the correlation with potency.

The activities exhibited by the entire set range over five natural log units (nearly 12 orders of magnitude). The equations thus can discriminate between quite potent and virtually inactive molecules. On this basis, the structural analysis derived from this admittedly limited set of nitrosamines may have utility as a theoretical screen for other untested nitrosamines.

A negative test is a result less than 0.01 revertant/nmole ($\ln R < -4.61$) (1). Results greater than 1.0 revertant/nmole ($\ln R > 0.00$) are positive to the degree of the value. McCann *et al.* (1) claimed a 90% correspondence between carcinogenicity and mutagenicity.

An examination of the equations reveals certain structure–activity relationships. The ability of the equations to discriminate between the subclasses is illustrated by a comparison of Compounds 2 and 8. Both have the same number of nonhydrogen atoms but vastly different activities. Both equations account for this difference. The activity of Compound 5 is correctly predicted to be less than its isostere, Compound 6. In Eq. 1, the $^0\chi$ values are the same for both; however, the $^1\chi^v$ value is less for Compound 5 because the valence connectivity delta assignment for oxygen in the ring is greater than the ring methylene delta in Compound 6. The cyclic molecules generally have a larger $^1\chi^v$ value than the noncyclic molecules and, therefore, are correctly predicted to be less active than cyclic molecules of about the same size.

This limited set of data provided a good relationship between a molecular connectivity description of structure and mutagenic potency (1) in the Ames test. This result may be a useful beginning to the development of theoretical prediction of mutagenic potential in discrete chemical classes of molecules. Examination of additional nitrosamines, similarly tested, should afford a constructive challenge to this approach. The intention is to examine other classes of mutagenic molecules using molecular connectivity.

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Determination of Plasma Hydrochlorothiazide Levels in Humans

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Abstract □ A method for determining plasma hydrochlorothiazide levels was developed with a sensitivity of 5 ng/ml. Accuracy and precision were demonstrated over the 5–648-ng/ml range by an overall recovery of 95 ± 8%. The detector response was linear for the 5–250-ng/ml range. The method was sufficiently sensitive for hydrochlorothiazide bioavailability studies and also was applicable for the determination of whole blood drug levels. Plasma levels in two subjects reached peak levels of 428 and 450 ng/ml at 2.5 and 2 hr, respectively, after a 50-mg dose. Whole blood levels at 3 hr after the same dose were 547 and 851 ng/ml and were approximately 2.5 times the 3-hr plasma levels.

Keyphrases □ Hydrochlorothiazide—GLC analysis in plasma □ GLC—analysis, hydrochlorothiazide in plasma □ Diuretics—hydrochlorothiazide, GLC analysis in plasma

The diuretic hydrochlorothiazide has been used clinically for a number of years. In spite of the availability of GLC (1, 2) and liquid chromatographic (3, 4) methods, only limited human plasma level data have been published. Plasma levels were reported (1) in four subjects, but levels past 4 hr (6 and 24 hr) were noted for only one subject. Plasma level–time curves were reported for a single subject (5), as was an average curve from eight subjects covering 9 hr following administration (6).

This paper reports individual plasma level data obtained in a study with two male volunteers. Plasma hydrochlorothiazide levels were determined using a modification of the GLC method reported previously (2).

EXPERIMENTAL

Procedure—Plasma (2 ml) was pipetted into a 12-ml polytef[1]-lined screw-capped centrifuge tube. The internal standard, the bromo analog of hydrochlorothiazide (6-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide), was added (200 μl of a 1-μg/ml solution in methanol), and the sample was mixed thoroughly on a vortex mixer[2]. Then methyl isobutyl ketone[3] (5 ml) was added, and the tightly closed tube was vigorously shaken horizontally on a platform shaker[4] for 20 min.

Following centrifugation for 20 min, 4 ml of the organic layer was transferred to a clean 12-ml centrifuge tube. Sodium hydroxide (2.5 ml, 0.1 *M*) was added, and the tightly closed tube was vigorously shaken for 20 min. Following centrifugation for 15 min, 2 ml of the aqueous layer was transferred to a clean 12-ml centrifuge tube. A solution of tetrahex-

[1] Teflon, E.I. du Pont de Nemours, Wilmington, Del.
[2] Scientific Industries Inc., Springfield, Mass.
[3] J. T. Baker, Phillipsburg, N.J.
[4] Eberbach Corp., Ann Arbor, Mich.

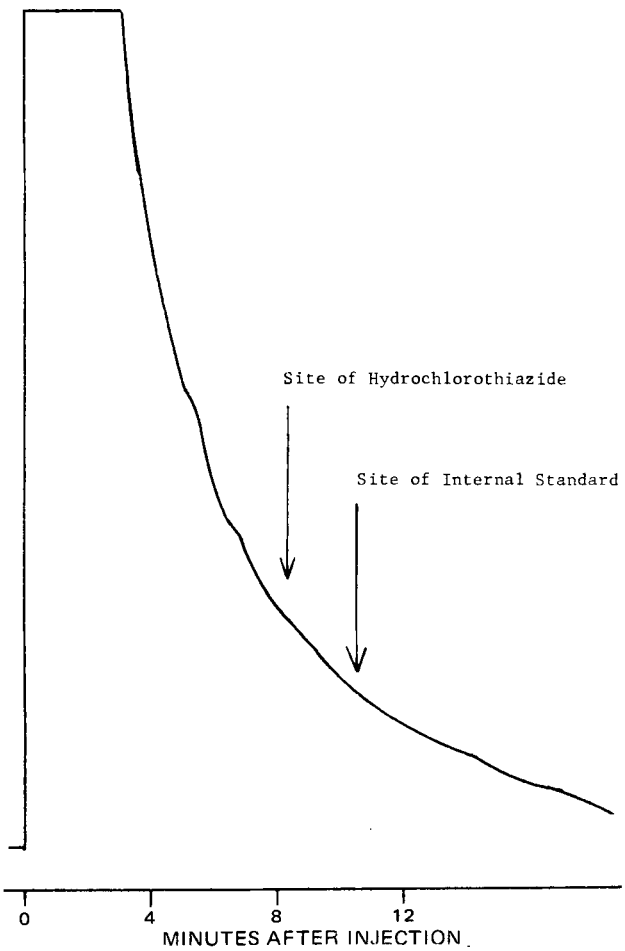

Figure 1—*Gas chromatogram of human plasma blank from a subject before hydrochlorothiazide administration.*

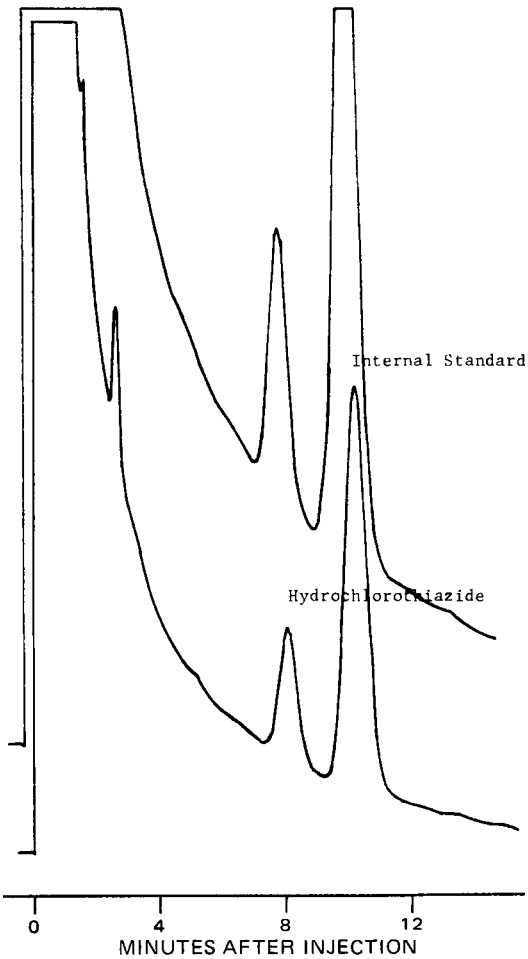

Figure 2—*Gas chromatogram of human plasma from a subject after hydrochlorothiazide administration (with internal standard added) using the dual-pen recorder technique.*

ylammonium hydrogen sulfate[5] (50 μl, 0.1 *M* in water, neutralized with sodium hydroxide) and 5 ml of 0.5 *M* methyl iodide in dichloromethane were added. The tightly closed tube was shaken horizontally for 30 min in a thermostatic heating block[6] (50°) mounted on a platform shaker.

Following centrifugation for 15 min, 4 ml of the organic layer was transferred to an 8-ml culture tube with a 6-mm o.d. × 10-mm long tip at the bottom; the solvent was then evaporated at room temperature under a nitrogen stream. A suitable volume of cyclohexane was added, and the closed tube was placed in an ultrasonic bath for 5 min. Following centrifugation for 15 min, 2 μl of the solution was injected into the gas chromatograph.

A gas chromatograph[7] with a linearized electron-capture detector[8] and a dual-pen recorder[9] were used. The recorder was run with both pens operating simultaneously: one with a 1-mv range, the second with a 2-mv range. A 0.9-m × 2-mm i.d. glass column was packed with 80–100-mesh 1% OV-225 on an acid-washed, dimethylchlorosilane-treated support[10] and was conditioned at 260° for 48 hr before use. The carrier gas was 95% argon–5% methane at a 50-ml/min flow rate. Oven, injection port, and detector temperatures were 260, 275, and 325°, respectively. The oven temperature was reduced to 200° overnight to increase the column life.

The hydrochlorothiazide concentration in a sample was determined by comparing the peak height ratio (hydrochlorothiazide derivative peak height/internal standard derivative peak height) to a standard curve of peak height ratio *versus* hydrochlorothiazide concentration. The standard curve was generated daily by carrying spiked plasma standards of several known concentrations through the procedure along with the samples.

Human Study—The subjects, two healthy white males, did not eat after dinner on the day prior to the study but were permitted to drink fluids without caffeine. Before leaving home on the day of the study, each subject drank at least 200 ml of water.

Control blood samples were obtained from each prior to the administration of one 50-mg hydrochlorothiazide tablet[11]. Subjects received 200 ml of water with their tablet and hourly thereafter for 8 hr. A light breakfast was served 2 hr after drug administration.

Blood samples (10 ml) were obtained at 30-min intervals for the first 3 hr and at 4, 6, 8, and 24 hr. All blood samples were collected in heparinized tubes. The 10-ml samples were centrifuged as soon as they were collected, and the separated plasma was frozen immediately. An additional 5-ml blood sample was obtained at 1, 2, and 3 hr. These samples were transferred to properly labeled containers and frozen.

RESULTS AND DISCUSSION

The method used for hydrochlorothiazide analysis in plasma differed from that of Lindstrom *et al.* (2) in three ways. First, the use of phosphate buffer was not necessary. Second, the use of the bromo analog of hydrochlorothiazide as the internal standard, instead of chlorthalidone, permitted the internal standard to be added directly to the whole blood sample before extraction. And, third, a more polar liquid phase (OV-225) was used in place of SE-30 for the chromatographic separation.

Typical gas chromatograms for human plasma from a male volunteer before and after oral administration of a 50-mg hydrochlorothiazide tablet are shown in Figs. 1 and 2, respectively. Derivatized hydrochlorothiazide, whether from blank plasma with authentic hydrochlorothiazide added or from plasma samples obtained following hydrochlorothiazide administration, afforded a well-defined peak with a retention time of ap-

[5] A. B. Hassle, Molndal, Sweden.
[6] Multi-Temp-Blok, Lab-Line Instruments, Melrose Park, Ill.
[7] Model 5710A, Hewlett-Packard, Avondale, Pa.
[8] Model 18713A, Hewlett-Packard, Avondale, Pa.
[9] Model A-25, Varian, Palo Alto, Calif.
[10] Supelcoport, Supelco, Inc., Bellefonte, Pa.

[11] Esidrix, Ciba-Geigy Corp.

Table I—Recovery of Hydrochlorothiazide from Plasma Samples [a]

Added Concentration, ng/ml	Found Individual Values, % of Added Concentration					
5	100	100	100	—	—	—
16	106	94	94	94	94	—
29	83	86	83	100	—	—
49	106	102	84	—	—	—
51	86	80	86	84	—	—
65	92	89	88	—	—	—
118	91	85	82	81	—	—
162	99	102	102	99	99	—
200	97	93	91	88	—	—
243	103	93	109	—	—	—
324	98	102	101	91	108	102
329	102	93	99	97	—	—
486	95	100	104	—	—	—
648	95	108	98	—	—	—
	Overall recovery ± *SD*			95 ± 8%		

[a] Prepared by adding known amounts of hydrochlorothiazide to control human plasma.

proximately 8 min. The derivatized internal standard also afforded a well-defined peak, its retention time being approximately 10.5 min.

Accuracy and Precision—Accuracy and precision were determined by calculating recovery as a percent of the added concentration for all samples over the range of hydrochlorothiazide levels from 5 to 648 ng/ml (Table I). The overall recovery of 95 ± 8% (*SD*) demonstrated good accuracy and precision.

Linearity—Standard curves, developed from plasma spiked with hydrochlorothiazide over the 5–250-ng/ml range, exhibited good linearity as demonstrated by a typical correlation coefficient of 0.998 for the linear regression line. The reliability of determining low concentrations of hydrochlorothiazide (25 ng/ml or less) can be increased by using a standard curve over the 5–75-ng/ml range.

Sensitivity—As seen in Table I, the method has good accuracy and precision at a plasma hydrochlorothiazide concentration as low as 5 ng/ml. Concentrations below this level have not been studied.

Stability—Samples were prepared by adding hydrochlorothiazide to control plasma to obtain concentrations of 15, 74, and 149 ng/ml. The samples were analyzed immediately and divided into aliquots which were then frozen. Analysis of duplicate samples of each concentration after 2 months of storage at −20° afforded average recoveries of 100, 99, and 98% for the three concentrations, respectively. These results demonstrate that hydrochlorothiazide is stable in plasma, under frozen storage conditions, for at least 2 months.

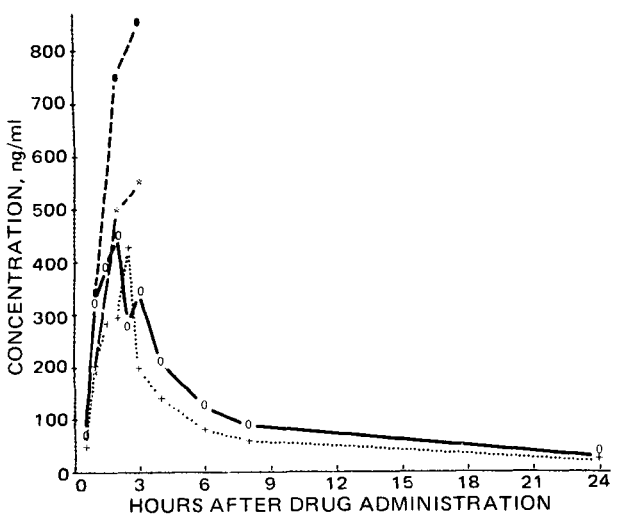

Figure 3—*Plasma (+ and O) and whole blood (* and ●) levels of hydrochlorothiazide following oral administration of one 50-mg commercial tablet to two volunteers.*

Table II—Plasma and Whole Blood Hydrochlorothiazide Levels following Oral Administration of 50 mg

Hours	Subject 1	Subject 2
	Plasma Levels, ng/ml	
0.5	46	69
1	201	320
1.5	282	387
2	294	450
2.5	428	276
3	199	342
4	140	205
6	80	120
8	58	83
24	18	24
	Blood Levels, ng/ml	
1	185	338
2	493	747
3	547	851

Clinical Study—Plasma and whole blood hydrochlorothiazide levels following oral administration of one 50-mg commercial hydrochlorothiazide tablet to two volunteers are summarized in Fig. 3 and Table II. Predose (zero-time) plasma specimens were devoid of any material at the hydrochlorothiazide retention time. Peak plasma levels were observed at 2.5 and 2 hr in Subjects 1 and 2, respectively. Plasma hydrochlorothiazide concentrations declined rapidly at first from peak values but slowed appreciably by 8 hr after administration. This latter decline was in agreement with the 13.1-hr half-life of the β-phase reported by Beermann *et al.* (5). Areas under these plasma level–time curves (0–24 hr) were 1811 and 2494 hr × ng/ml for Subjects 1 and 2, respectively.

Whole blood levels were monitored at 1, 2, and 3 hr after administration. These levels were highest at 3 hr. Blood and plasma levels were comparable at 1 hr after administration; but by 3 hr after administration, the blood levels were approximately 2.5 times the plasma levels. This phenomenon of markedly higher whole blood than plasma levels was reported by Beermann *et al.* (5), who found levels of radioactivity in red blood cells 3.5 times those observed in plasma. Similarly, whole blood levels of chlorthalidone and a carbonic anhydrase inhibitor, 2-amino-4-phenylsulfonylbenzenesulfonamide, were reported to be 6–35 times as high as the respective plasma levels in humans (7, 8). The mechanism underlying these findings has not been elucidated.

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