Diazoxide Blood Levels in Man

By S. SYMCHOWICZ, L. WINSTON, J. BLACK*, M. SMITH, B. CALESNICK, and I. I. A. TABACHNICK

Blood diazoxide levels were measured in six adult human males at various times after i.v. drug administration. On the basis of these results, the diazoxide half-life in blood was determined as 28.0 ± 8.3 hr. The presence of diazoxide in blood was verified by thin-layer chromatography (TLC) and by its U.V. spectrum. This communication describes a simple spectrophotometric quantitative method and a useful TLC method for determination of diazoxide in biological fluids.

DIAZOXIDE is a nondiuretic benzothiadiazine which was originally developed for the treatment of hypertension. Early observations showed that diazoxide produced hyperglycemia in animals and man (1–5) which limited its use as an antihypertensive agent in man (6–8). However, the hyperglycemic effect of diazoxide is being increasingly utilized for treatment of patients with severe hypoglycemia (9–16).

A relationship was shown (17) between diazoxide dosage forms, dissolution rates, and blood levels. This report presents data on blood half-life of diazoxide in man and describes the methods for diazoxide analysis in biological fluids.

MATERIALS AND METHODS

Diazoxide was given intravenously at 5 mg./Kg. to six young adult males (weighing between 66 and 88 Kg.) in good physical health with no known cardiovascular or renal disease, and no abnormal glucose tolerance curve or family history of diabetes. The sterile diazoxide solution contained the equivalent of 300 mg. of the drug in 20 ml. of water adjusted to pH 11.4 with NaOH. Blood samples were drawn at 0, 5, 15, 30, and 60 min. and 2, 4, 6, 12, 24, 48, and 72 hr. after injection of diazoxide which lasted 2 min. All morning samples were taken before breakfast. Strenuous physical activity was avoided and normal diet was maintained.

The diazoxide blood levels were determined quantitatively as follows: 1 ml. of distilled water and 2 ml. of whole blood were mixed vigorously in a 15-ml. glass-stoppered centrifuge tube for 1 min. with 4 ml. of ethyl acetate. After brief centrifugation (2–3 min. at 1,300 r.p.m., International centrifuge PR-2), 2.5 ml. of the organic phase was transferred to another centrifuge tube containing 2.5 ml. of 4% sodium carbonate solution. The contents of the tube were mixed thoroughly (1–2 min.) and after centrifugation, the clear, aqueous phase was transferred to a quartz cell (1 cm. light path). The absorbance was read at 280 mμ in a Beckman DU spectrophotometer. A 4% sodium carbonate solution was used to set the instrument to zero absorbance. All samples were corrected for individual "blank" readings (zero hour sample).

The diazoxide blood concentration was determined from a standard curve. A linear relationship was obtained with diazoxide standards over a wide range of drug concentration (1–25 mcg./ml.). Studies with blood, plasma, and water, by our procedure, yielded on the average about 80% recovery of the drug.

Thin-layer chromatography was employed as an additional tool for identification of diazoxide and for its semiquantitative estimation. One to two milliliters of plasma was extracted with 4 ml. of ethyl acetate. The organic solvent was concentrated to a small volume (under nitrogen or on a rotating evaporator) and then applied quantitatively to Silica Gel F 254 precoated plates (Brinkmann Co.) which were developed with benzene–methanol (1:4) for 30–45 min. Diazoxide was visualized as a purple spot on the chromatographic plates with a short-wave U.V. lamp.

The half-life of diazoxide was estimated from the mathematical function $\log \hat{y} = a + bt$, where $\hat{y}$ = drug concentration (mcg./ml.), t = time in hours, a = y intercept (value of y when $t = 0$), and b = slope.

The function was fitted by the method of "least squares" (18).

RESULTS AND DISCUSSION

Table I summarizes the results obtained by spectrophotometric analysis of diazoxide blood levels at various time intervals. The half-lives were estimated from the function $\log y = a + bt$ (see *Methods*) and they range from 20.9 hr. (subject 1) to 34.7 hr. (subject 2). All slopes (b) are significantly different from 0 ($p < 0.01$, Student t test).

The mean blood half-life in man was estimated after testing the homogeneity of the regression lines (derived from Table I) by analysis of covariance. A weighted regression estimate for diazoxide is given by the function; $\log \hat{y} = 1.0483 - 0.0110t$ (Fig. 1) from which the half-life was derived as 28.0 + 8.3 hr. (95% confidence interval). The individual drug blood concentrations at various time intervals are shown in Fig. 1.

TABLE I—HALF-LIFE OF DIAZOXIDE IN HUMAN BLOOD

Subject	a	b	Estimated[a] Half-Life, hr.
1	0.9928	0.0142	20.9
2	0.9701	0.0087	34.7
3	1.1083	0.0136	22.2
4	1.0584	0.0094	32.1
5	1.0989	0.0096	31.4
6	1.1087	0.0107	28.2

[a] Derived from function $\log \hat{y} = a + bt$, where a = log mcg./ml. at time 0 and b = (rate of change per unit change in time), log mcg./ml.

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* Present address: Warner-Lambert Research Institute, Morris Plains, NJ 07950

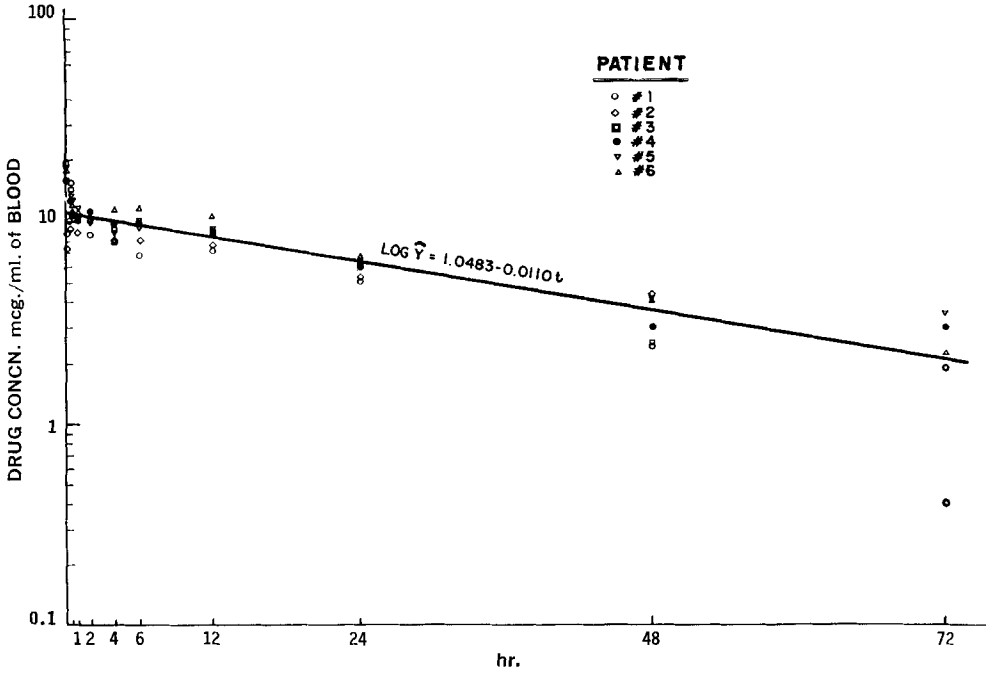

Fig. 1—Least square regression line for blood half-life evaluation in man.

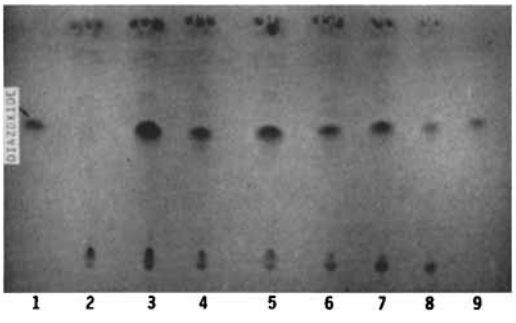

Fig. 2—TLC pattern of diazoxide extracted from plasma (photographed under U.V. light). Samples 1 and 9 show the position of diazoxide standards (5 mcg.). Sample 2 represents the pattern of untreated (0 hr.) plasma. Samples 3 and 4 are from subject 1, at 30 min. and 24 hr., respectively. Samples 5 and 6 are corresponding time intervals for subject 2 and samples 7 and 8 for subject 6.

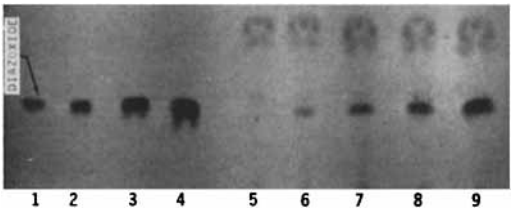

Fig. 3—TLC analysis of diazoxide recovered from plasma (photographed under U. V. light). Samples 1, 2, 3, and 4 are diazoxide standards spotted directly at concentrations of 5, 10, 20, and 40 mcg., respectively. Samples 6, 7, 8, and 9 represent the same respective diazoxide concentrations recovered from plasma. Sample 5 is plasma control (0 hr.).

To identify the material read at 280 mμ, particularly at the later time intervals, thin-layer chromatography was employed. Thirty-minute and 24-hr. plasma samples from subjects 1, 2, and 6 were extracted with ethyl acetate (see *Methods*) and chromatographed. The results in Fig. 2 clearly show that diazoxide is the only major component visible in plasma when compared with control (0 hr., spot 2). Samples from each subject show, as expected, more intense diazoxide spots at the earlier time interval. Other spots visible under U.V. light (Fig. 2) correspond to unidentified constituents of plasma (compare with control plasma, spot 2). Samples 1 and 9 are the diazoxide standards (5 mcg.).

Further identification of the diazoxide spot visible upon chromatography of plasma samples was obtained as follows. The areas on the glass plate (samples 2, 3, and 4, Fig. 2) were scraped out, and extracted with ethyl acetate, which was then extracted with 4% sodium carbonate. The U.V. spectrum of the sodium carbonate solution was recorded on a Perkin-Elmer recording spectrometer and compared with 5 mcg. of a chromatographed standard diazoxide solution (sample 1, Fig. 2) subjected to the same procedure. Both the standard and plasma samples showed identical U.V. characteristics, thus verifying the identity of the diazoxide spot on TLC plates.

The TLC procedure in this investigation is quite sensitive for diazoxide and gives good plasma recoveries. Figure 3 shows that the intensities of spots are proportional to the amounts (5, 10, 20, and 40 mcg.) of diazoxide standard spotted. Similar results were obtained by adding the same amounts of diazoxide standard to 1-ml. plasma samples and subjecting them to the described extraction procedure (samples 6–9, Fig. 3). Sample 5 is plasma control (no drug added).

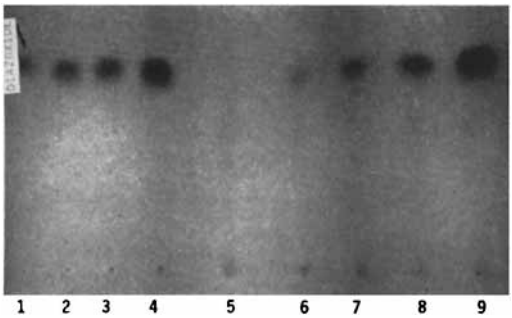

Fig. 4—TLC analysis of diazoxide recovered from urine (photographed under U. V. light). Samples 1, 2, 3, and 4 represent diazoxide standards 5, 10, 20, and 40 mcg., respectively. Samples 5, 6, 7, 8, and 9 represent diazoxide recovered from urine to which 0,5, 10, 20, and 40 mcg. of the drug was added, respectively.

Although no attempt was made to analyze diazoxide levels in urine, the TLC procedure is applicable to urine as well. It is recommended, however, that after extraction of urine with ethyl acetate, the organic solvent be washed with 2.5 ml. of 4% sodium bicarbonate solution before it is concentrated for chromatography. This will remove many interfering materials present in urine and give good diazoxide recoveries. Samples 6–9 (Fig. 4) present results obtained on recovery from urine of 5, 10, 20, and 40 mcg. of diazoxide, respectively. The size and intensity of diazoxide spots compare favorably (assuming some losses due to extraction procedure) with that obtained by direct chromatography of respective diazoxide standards (samples 1 4). Sample 5 represents control urine with no drug added.

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Proteinaceous Antitumor Substances from Plants III. *Caesalpinia gilliesii* (*Leguminosae*)

By A. ULUBELEN*, W. F. MCCAUGHEY†, and J. R. COLE

Preliminary screening of the aqueous extracts of *Caesalpinia gilliesii* have shown activity against sarcoma 180 in mice and Walker 256 in Sprague rats. The isolation, purification, and partial characterization of six proteinaceous materials are reported.

As a result of a routine screen of Southwestern plants for potential antitumor activity, the aqueous extracts of *Caesalpinia gilleisii*[1] (Wall) demonstrated activity toward the sarcoma 180 test system in mice.[2] This screening program was carried out by the Cancer Chemotherapy National Service Center, Bethesda, Md. Further fractionation has resulted in activity against Walker 256 (5WM) in Sprague rats as well as the sarcoma 180 (3SA) test system in mice.

The plant is an odoriferous, glandular shrub 2.3 M. tall, collected near Greaterville, Ariz., where it has become naturalized in a pasture.

EXPERIMENTAL

Ten Kg. of the dry pods of *C. gilliesii* was extracted with approximately 15 L. of water at room temperature. The aqueous extract was lyophilized in a Repp Industries model 15 sublimator and then washed with MeOH and EtOH. The yield was 760 Gm. One-hundred grams of this crude compound was added to 1 L. of water at room temperature. The insoluble part was separated by centrifuge and discarded.

The aqueous solution was extracted with 5 × 200 ml. of ether to remove fatty materials, and increased in volume threefold by the addition of 95% ethanol. A light-colored precipitate appeared and was separated by centrifuge, dissolved in water, and dialyzed against running water at room tem-

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Previous paper: Ulubelen, A., and Cole, J. R., *J. Pharm. Sci.*, **55**, 1368(1966).
*Present address: College of Pharmacy, University of Istanbul, Istanbul, Turkey.
[1] Identification confirmed by Robert Barr, College of Pharmacy, and Dr. Charles Mason, Botany Department, University of Arizona, Tucson. A reference specimen was also deposited.
[2] Preparation of the preliminary extraction was carried out by Dr. M. E. Caldwell.