Correlation of Amount of Metabolite Excreted and Its Excretion Rate with Available Surface Area of Tolbutamide in Dosage Form

By EINO NELSON, STUART LONG, and JOHN G. WAGNER

Tolbutamide was administered to normal human test subjects in the form of thin cylindrical disks having an initial surface area of 6.60 cm.² per 0.5 Gm. dose and in the form of spherical granules prepared by coating spherical inert core granules with this drug. The initial surface areas of the granules used were 36.9, 63.1, and 98.6 cm.² per 0.5 Gm. dose of tolbutamide. Excretion rate of carboxytolbutamide, the metabolite of tolbutamide, was determined following dose ingestion. Perfect rank-order correlation was shown between per cent of a dose as metabolite excreted in the urine in 48 hours and surface area of tolbutamide in the dosage form. Likewise, there was perfect rank-order correlation between mean maximum excretion rate of carboxytolbutamide and surface area of tolbutamide in the dosage form.

WHILE THERE is now good recognition of the qualitative aspects of the effect of surface area of drug on absorbability, little work has been done on the quantitative aspects of this factor, although the effect of surface area on the absorption of tetracycline and several of its salts has been previously studied in cases of solution rate-limited absorption and nonsolution rate-limited absorption (1, 2). This communication reports the results of studies conducted using dosage forms of the antidiabetic drug tolbutamide,[1] with known surface areas in which a direct relationship was shown between either mean maximum excretion rate of carboxytolbutamide,[2] the metabolite of tolbutamide, or percentage of this metabolite recovered in the urine in 48 hours and average surface area of tolbutamide when drug was taken as thin cylindrical disks or spherical granules.

EXPERIMENTAL

Dosage Forms.—Thin cylindrical disks, 0.168 cm. in thickness and 1.28 cm. in diameter, were prepared by compressing tolbutamide with 1% calcium stearate in a single punch tablet compressing machine. The apparent density of the disks was 1.16 Gm./cm.³. Each disk contained 250 mg. of tolbutamide; hence, two disks contained a 0.5-Gm. dose of tolbutamide.

Three lots of spherical granules of tolbutamide were prepared in a manner similar to that previously described (3), except that no external coating was applied. By varying the size of the core granules and the thickness of the coated tolbutamide layer, the surface areas per 0.5 Gm. dose of tolbutamide were altered from one lot of granules to the next.

Particle size variation was estimated by measuring the diameters of 100 individual granules of each lot with a micrometer. The average and median diameters, the standard deviations, and the coefficients of variation are shown in Table I. Percentage of granules by number less than diameter against diameter is plotted on normal probability graph paper in Fig. 1. Approximately 95% of the granules of each lot gave linear plots which indicated this fraction approximates the normal Gaussian distribution. There was some skewness to the left, *i.e.*, toward smaller diameters. The medians, standard deviations, and coefficients of variation were estimated from the linear portions of the plots shown in Fig. 1, and these are reported in Table I also. In a similar manner, particle size variation of the core granules was estimated. The variation in particle size of the core granules was of the same order of magnitude as the particle size variation of the coated granules. Wachtel and LaMer (4) reported an average coefficient of variation of 12.6% for so-called monodisperse emulsions and an average coefficient of variation of 10% for aerosols. Hence, the smaller coefficients of variation of the core and coated granules, approximately 4–6%, indicate very narrow particle size ranges.

More accurate estimates of average diameters of the core and coated granules were obtained by measuring about 1,000 granules of each lot. This was done by lining up the granules side by side in a trough in groups of 110 to 130 and measuring the distance from the first to the terminal granule. The average diameters determined by this method are shown in Table II and are within 1 to 3% of the average diameters obtained with the 100 individually measured granules of each lot. From the average diameters and weights shown in Table II, the apparent densities of the coated tolbutamide layers on the granules were calculated to be 0.997, 0.930, and 1.02 Gm./cm.³ for granules A, B, and C, respectively.

Calculation of Surface Areas and Doses.—Each lot of granules was considered sufficiently homogeneous and of particle size range narrow enough to estimate the surface area from the average diameters of the core granule and the coated granules. To illustrate the method of calculation, the data for granules C (Table II) are used. The surface area of an average coated granule was π (0.1195)² or 0.0449

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[1] Tolbutamide is 1-butyl-3-*p*-tolylsulfonylurea.

[2] Carboxytolbutamide is *p*-(butylcarbamoyl) sulfamoylbenzoic acid.

TABLE I.—PARTICLE SIZE VARIATION OF THE COATED GRANULES

	Granules A	Granules B	Granules C
Based on 100 Granules of Each Lot:			
Av. (cm.)	0.129	0.135	0.117
S.D. (cm.)	0.0072	0.0076	0.0051
Coefficient of variation (%)	5.6	5.6	4.4
Median (cm.)	0.129	0.136	0.117
Based on Linear Portion of Normal Probability Plot:			
Median (cm.)	0.129	0.137	0.117
S.D. (cm.)	0.0066	0.0064	0.0046
Coefficient of variation (%)	5.1	4.7	3.9

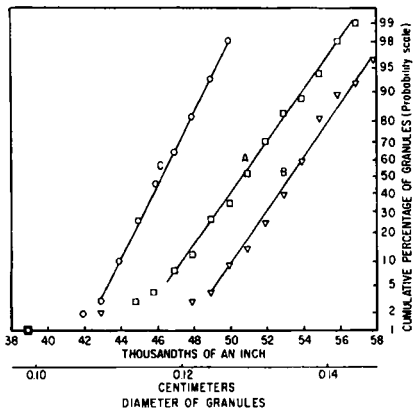


Fig. 1.—Particle size distributions of the granules. Key: O, granules C; □, granules A; ▽, granules B.

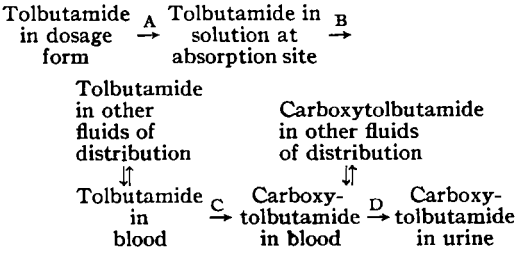
cm.²; hence, the surface area of 2195 granules was 98.6 cm.². The surface area of an average core granule was π (0.1075)² or 0.0363 cm.²; hence, the surface area of 2195 granules was 79.7 cm.². The average surface area during dissolution of the coated layer was then (98.6 + 79.7)/2 or 89.1 cm.² per 0.5-Gm. dose of tolbutamide. The weight of coated granules required was obtained by dividing the dose (in milligrams) by the potency or 500/157 or 3.185 Gm. The coated granules weighed 14.5103 Gm. per 10,000 or an average of 1.45103 mg./granule. The number of granules administered was therefore 3185/1.45103 or 2195 granules. The corresponding calculated quantites for granules lots A and B are shown in Table II.

The surface area of the disks calculated from their dimensions was 3.30 cm.²/disk or 6.60 cm.² per 0.5-Gm. dose of tolbutamide. Since their final surface area after dissolution would be theoretically zero, their average surface area during dissolution would be 3.30 cm.² per 0.5 Gm. of tolbutamide.

Conduction of Tests.—Drug in 0.5-Gm. doses was ingested orally by *normal* human test subjects after overnight fast, and no food was taken until 1 hour after ingestion of the dose. The doses of the drug as spherical granules were contained in an appropriate number of hard gelatin capsules. Urine was collected at 2-hour intervals for the first 12 hours and then in pooled 12-hour collections for 48 or 60 hours. The designations (by initial) of sex, age, and weight in kilograms of the test subjects were, respectively, E-M-41-77, C-F-30-57, and P-M-31-72. The various preparations were taken serially in the order disks and granules A, B, and C, respectively. At least 1 month elapsed between tests.

Analytical Method.—Suitable diluted urine aliquots were assayed for carboxytolbutamide by the method of Nelson *et al.* (5). Suitable blank and binding corrections were made on the initial analytical values as prescribed in the assay.

THEORY



It is known from previous work (6, 7) that (*a*) B, C, and D are or could be expected to be first-order processes within experimental error; (*b*) process D is about 12 times more rapid than process C; (*c*) process B is at least 10 times more rapid than process C; and (*d*) tolbutamide is nearly quantitatively oxidized to carboxytolbutamide (*i.e.*, $f \cong 1$).

Hence, assuming that tolbutamide entering the blood during the absorption process rapidly equilibrates with the blood and other fluids of distribution, one would expect that the rate of excretion of the metabolite, carboxytolbutamide, would be greatly influenced by the rate(s) of process A. Since rate of dissolution is theoretically directly proportional to surface area, one would expect the rate of excretion of the metabolite to be related to the surface area of tolbutamide in the administered dosage form. Also, since one would expect the dosage form to be at the absorption sites in the gastrointestinal

TABLE II.—AVERAGE DIAMETERS, AVERAGE WEIGHTS, POTENCIES, DOSES, AND SURFACE AREAS OF GRANULES

	Granules A	Granules B	Granules C
Av. diam. of coated granules (cm.)	0.1326	0.1368	0.1195
Av. diam. of core granules (cm.)	0.0937	0.1151	0.1075
Av. wt. of coated granules (Gm. per 10,000 granules)	15.9875	19.8227	14.5103
Av. wt. of core granules (Gm. per 10,000 granules)	8.5312	14.7800	12.0109
Potency (mg. tolbutamide per Gm. of granules)	467	235	157
Wt. of granules equiv. to 0.5 Gm. tolbutamide (Gm.)	1.071	2.131	3.185
No. of granules equiv. to 0.5 Gm. tolbutamide	669	1075	2195
Total surface areas for No. of granules equiv. to 0.5 Gm. of tolbutamide			
Coated granules	36.9	63.1	98.6
Core granules	18.5	44.7	79.7
Av. during dissolution of coated layer	27.7	53.9	89.1

TABLE III.—AMOUNTS OF CARBOXYTOLBUTAMIDE (mg.) EXCRETED BY TEST SUBJECTS AFTER INGESTION OF 500-mg. DOSES OF TOLBUTAMIDE IN SEVERAL DOSAGE FORMS[a]

Subject	Time, Hr. 2	4	6	8	10	12	24	36	48	60
Thin Cylindrical Disks D, 6.6 cm.²/0.5 Gm. Dose Tolbutamide										
E[b]	10	30	47	61	71	84	124	...	219	
C[b]	2	9	21	33	46	54	93	...	124	
P	1	12	24	34	43	49	89	...	140	
Mean	4	17	31	43	53	62	102	...	161	
Granules A, 27.7 cm.²/0.5 Gm. Dose Tolbutamide										
E	10	19	38	73	116	126	211	246	269	...
C	4	21	45	78	99	120	187	230	252	...
P	0	17	37	62	86	104	171	209	229	...
Mean	5	19	40	71	100	117	189	228	250	...
Granules B, 53.9 cm.²/0.5 Gm. Dose Tolbutamide										
E	6	23	52	86	115	146	191	260	287	319
C	8	87	163	222	276	312	449	486	500	516
P	6	41	79	120	156	184	322	402	432	465
Mean	7	50	98	143	182	214	321	382	406	433
Granules C, 107.6 cm.²/0.5 Gm. Dose Tolbutamide										
E	43	121	203	272	333	379	510	556	580	599
C	32	137	222	294	348	387	534	573	590	590
P	22	72	119	158	199	227	366	448	480	504
Mean	32	110	181	241	293	331	470	526	550	564

[a] Theoretical maximum recovery, 555 mg. [b] Data for these subjects are also reported in another communication (6).

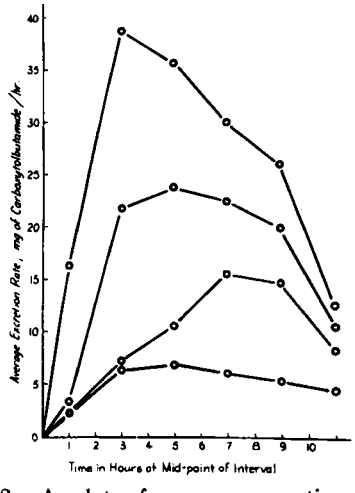


Fig. 2.—A plot of average excretion rate of metabolite against time. The curves from top to bottom are for granules C, granules B, granules A, and the disks, respectively.

tract for some finite time or range of time, the amount of metabolite excreted in a given time should be related to the surface area of tolbutamide in the administered dose. Due to the complexity of the biological situation, as illustrated by the model above, it is not possible to estimate quantitatively the rate of process A as a function of time from the rate of appearance of the metabolite in the urine. The amount of metabolite in the urine at a given time will be a function of the volumes of many of the "compartments" involved and many of the rate and/or permeability constants involved. We tested the feasibility of making rank-order correlations between extent and rate of excretion of carboxytolbutamide and available surface area of tolbutamide in the administered dosage form.

RESULTS

Amounts of carboxytolbutamide excreted in various times are given in Table III. Figure 2 is a plot of average excretion rate of carboxytolbutamide (milligrams per hour) against time at the midpoint

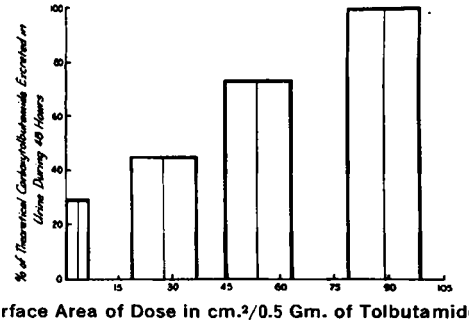


Fig. 3.—A perfect rank-order correlation of per cent of theoretical metabolite excreted in the urine in 48 hours with surface area of tolbutamide in the dosage form. The center line in each bar gives the average surface area during dissoluton.

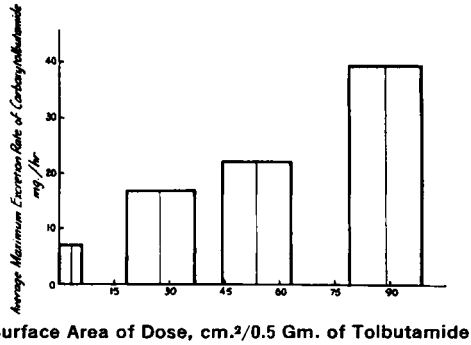


Fig. 4.—A perfect rank-order correlation of average maximum excretion rate of metabolite in the urine with surface area of tolbutamide in the dosage form. The center line in each bar gives the average surface area during dissolution.

of the collection intervals over which the rates were calculated. Such excretion rate-time plots are much more sensitive and show curvature not nearly as evident on a cumulative amount excreted *versus* time plot.

Figure 3 shows perfect rank-order correlation of per cent of theoretical carboxytolbutamide excreted in the urine in 48 hours with surface area of tolbutamide in the dosage form.

Figure 4 shows a perfect rank-order correlation of average maximum excretion rate of carboxytolbutamide in the urine with surface area of tolbutamide in the dosage form.

These results show that in normal human subjects available surface area of tolbutamide in the dosage form can have a pronounced effect on extent and rate of excretion of the metabolite, carboxytolbutamide, in the urine. Indirectly, they show that available surface area of tolbutamide in the dosage form can influence the rate and extent of absorption of tolbutamide if the surface area is restricted in the range studied.

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Alkaloids of *Vinca rosea* Linn. (*Catharanthus roseus* G. Don) XXIV

Vinaspine, Vincathicine, Rovidine, Desacetyl VLB, and Vinaphamine

By GORDON H. SVOBODA and ALBERT J. BARNES, JR.

The continued phytochemical investigation of this pantropical plant has resulted in obtaining vinaspine and vinaphamine as free bases and vincathicine, rovidine, and desacetyl VLB as the sulfates. The total number of alkaloids obtained from this plant, utilizing selective extraction, column chromatography, and gradient pH techniques, is now 49.

This investigation was pursued in an effort to elucidate the alkaloid composition of this pantropical plant as completely as possible (1). Occasionally, there is a spillover of leurocristine[1] (and leurosidine) into the B fraction. This investigation centers on the chloroform eluate of so-called post-leurocristine B fractions.

Rechromatography of the benzene-soluble material from the above fractions on deactivated alumina yielded only the known alkaloid perivine directly. Application of the gradient pH technique to each individual fraction yielded the new alkaloid vinaspine only as the base; while vincathicine, rovidine, and desacetyl VLB were obtained only as sulfates. A new alkaloid, vinaphamine, was obtained from crude amorphous leurosidine-containing material from the B fraction.

These new alkaloids are listed in Table I,

Table I.—New Alkaloids from *Vinca rosea* Linn.

Name	M. p., °C.	pK'a in 33% DMF	U.V. $\lambda_{max.}^{EtOH}$ mμ
Vinaspine	235–238	7.85	225, 281, 289
Vincathicine (sulfate)	>320 dec.	5.10, 7.05	231, 264, 300
Rovidine (sulfate)	>320 dec.	4.82, 6.95	214, 265, 286
Desacetyl VLB (sulfate)	>320 dec.	5.40, 6.90	214, 266, 294
Vinaphamine	229–235	5.15, 7.0	214, 262, 292

along with certain pertinent physical data. Their infrared spectra are reproduced separately (Figs. 1–4) as additional aids to their identification.

EXPERIMENTAL AND DISCUSSION[2,3]

Rechromatography of 3.659 Kg. of the chloroform eluate of post-leurocristine material from the B fraction from 90% leaf in benzene on 120 Kg. of deactivated alumina yielded 29.580 Gm. of perivine

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[1] The A.M.A. Council on Drugs has approved vinblastine, vinleurosine, vincristine, and vinrosidine as generic names for the four oncolytic alkaloids vincaleukoblastine (VLB), leurosine, leurocristine, and leurosidine, respectively. VLB is marketed as Velban (vinblastine sulfate), and leurocristine is marketed as Oncovin (vincristine sulfate) by Eli Lilly and Co., Indianapolis, Ind.

[2] For the sake of brevity, experimental techniques repeated from earlier work (2, 3) are not described.

[3] Melting points were determined on a Kofler microstage. Ultraviolet absorption spectra were obtained using a Cary model 14 spectrophotometer; infrared spectra with a Perkin-Elmer model 21 double beam recording infrared spectrophotometer; NMR spectra with a Varian Associates 60-megacycle spectrometer. A standard Norelco powder camera, 114.6 mm. in diameter, was used in the X-ray examination.