A second major reason for believing that particle size reduction was not achieved in the eutectic is provided by the second rate constant, which approximated the rate constant for the 50–60 mesh APAP. This result suggests that the urea is leached from the mixed eutectic particle leaving a matrix of APAP with an effective surface area comparable to that of a 50–60 mesh particle of APAP. Therefore, the value of eutectic formation as a means of enhancing dissolution rate still remains somewhat dubious. An enhancement of dissolution rate by virtue of simple eutectic formation alone is yet to be demonstrated.

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Increasing Dissolution Rates and Gastrointestinal Absorption of Drugs *Via* Solid Solutions and Eutectic Mixtures III

Experimental Evaluation of Griseofulvin–Succinic Acid Solid Solution

By ARTHUR H. GOLDBERG*, MILO GIBALDI, and JOSEPH L. KANIG

The phase diagram for the griseofulvin and succinic acid system showed a eutectic mixture with considerable solid solubility of griseofulvin in succinic acid. Evaluation of the dissolution rates of critical samples indicated that the solid solution dissolved 6.5–7 times faster than the pure material.

THE UTILIZATION of finely subdivided or micronized particles as a means of increasing the rate of dissolution of a drug has been considered frequently (1–4). Various methods of achieving particle size reduction have been reviewed by Levy (5). Sekiguchi *et al.* (6, 7) have suggested that particle size reduction, as a means of increasing gastrointestinal absorption of a drug, may be achieved through eutectic formation between a poorly soluble drug and a rapidly soluble carrier. Goldberg *et al.* (3, 4) have proposed that the increased absorption and dissolution rates found by Sekiguchi *et al.* were a function of the solid solutions present in the samples tested, rather than eutectic formation *per se*. Goldberg *et al.* (3) have also noted that the dissolution rate of a poorly soluble drug from a solid solution with a soluble carrier should be considerably faster than any other physical form of the drug, including the soluble eutectic and micronized forms. The purpose of this study was to evaluate experimentally the role of solid solutions in increasing dissolution rates.

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EXPERIMENTAL

The experimental procedures for selection of drugs and carriers, including the initial screening techniques, were reported previously (4). The system selected for this investigation consisted of the highly insoluble antifungal agent, griseofulvin,[1] with succinic acid as the carrier. The phase diagram for the binary system was prepared by the microthermal technique (4).

To determine any possible interaction between drug and carrier in aqueous solution, solubility studies were undertaken. An excess amount of drug was placed in 60-ml. test tubes equipped with screw caps and containing 30 ml. of an aqueous solution of the carrier in varying concentrations. The contents of each tube were equilibrated at 37° in a Gyrotory incubator shaker.[2]

At the end of this period, 1-ml. samples were withdrawn by means of a filter pipet. The griseo-

[1] Generously supplied by the Schering Corp., Bloomfield, N. J.
[2] Model G-25, New Brunswick Scientific Co., New Brunswick, N. J.

TABLE I.—COMPOSITION OF GRISEOFULVIN SAMPLES USED IN DISSOLUTION STUDIES

Sample	Compn.	Amt., mg.	Form	Particle Size, μ
1	Griseofulvin[a]	10.0	Crystalline	250–300
2	Griseofulvin	10.0	Eutectic	250–300
	Succinic acid	5.2		
3	Griseofulvin	10.0	Physically mixed	250–300
	Succinic acid	5.2		250–300
4	Griseofulvin	10.0	Solid soln.	250–300
	Succinic acid	40.0		
5	Griseofulvin	10.0	Physically mixed	250–300
	Succinic acid	40.0		250–300
6	Griseofulvin	10.0	Micronized	1–10

[a] Particle size enlargement of pure griseofulvin was obtained by crystallization from methanol. All other samples were fused. Griseofulvin, however, showed signs of decomposition upon heating to its melting point.

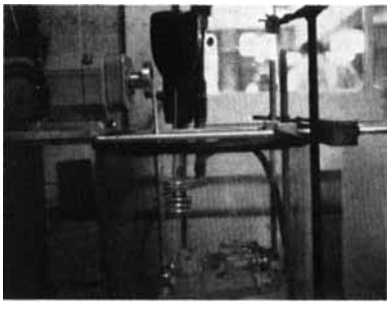


Fig. 1.—Oscillating bottle apparatus used for determining dissolution rates.

fulvin–succinic acid sample was diluted to 10 ml. with methanol and assayed spectrophotometrically at 292 mμ using a recording spectrophotometer.[3] A blank of 10% water in methanol was employed. The absorbance was read and the amount of griseofulvin present was determined by dividing the absorbance by the slope (obtained by the method of least squares) of a previously constructed Beer's law curve. Preliminary investigation had indicated that succinic acid did not interfere with the assay procedure. No shift in the absorbance peak was observed and solutions containing succinic acid gave the same absorbance as solutions containing an equal quantity of griseofulvin alone in the solvent.

The dissolution rate studies were conducted by 2 different methods. The apparatus used for one study is shown in Fig. 1. The various samples listed in Table I were screened, weighed, and placed into 10-ml. multiple-dose vials, sealed with an elastomer stopper, and capped with a metal ferrule having a hole in the center, exposing a portion of the top of the elastomer. Ten milliliters of water at 37° was injected by syringe through the stopper at time zero. The vial was immediately placed on the oscillating platform assembly in the water bath. The platform, in motion, described a 15° arc, 14 times per minute. Destructive sampling was used for the assays at 3, 5, 10, 15, and 20 min. after introduction. A sample was withdrawn by means of a syringe and rapidly filtered under vacuum using a Millipore[4] filter assembly. A 2-ml. sample of the filtrate was diluted to 10 ml. with methanol and assayed spectrophotometrically as previously described. A minimum of 2 experiments were conducted with each sample at each time interval.

The second method employed for evaluating the comparative dissolution rates was the tape method (8). This procedure involves accurately weighing and quantitatively transferring the screened material to be tested to a taut, adhesive surface and, in turn, introducing the resulting monoparticulate layer to the dissolution medium. Four hundred milliliters of distilled water, maintained at 37°, served as the dissolution fluid. Stirring rate was maintained at 130 r.p.m. by means of a constant speed motor. The stir paddle employed was 2 times the width of the stir paddle previously reported (8). The increased speed and wider stir paddle was necessitated by the extremely low solubility of griseofulvin.

Samples 1, 2, 4, and 5 of Table I were evaluated by the tape method. Sampling times were 0.5, 1.5, 3.0, 5.0, and 10 min. At each time interval 10-ml. samples were withdrawn by means of a filter pipet and immediately replaced with 10 ml. of dis-

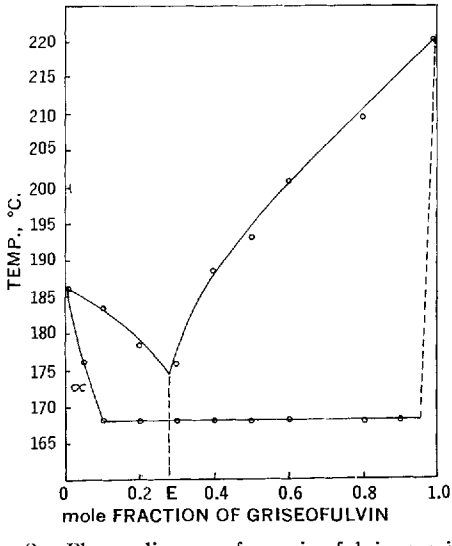


Fig. 2.—Phase diagram for griseofulvin–succinic acid mixtures obtained by microthermal techniques.

[3] Model DB, Beckman Instrument Co., Mountainside, N. J.

[4] Filter No. HA, 0.45 μ, white, plain, 25 mm. Millipore, Inc., Bedford, Mass.

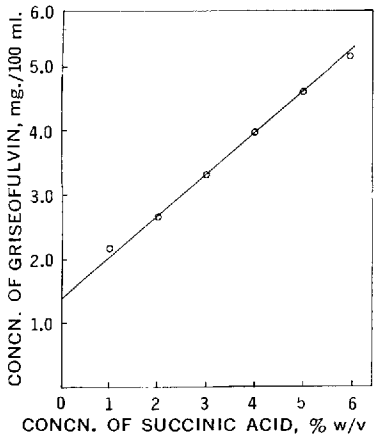


Fig. 3.—Effect of succinic acid on the aqueous solubility of griseofulvin.

tilled water preheated to 37°. Griseofulvin concentration was determined spectrophotometrically. All values were corrected to account for drug removed by prior sampling.

RESULTS AND DISCUSSION

The phase diagram obtained with the griseofulvin–succinic acid mixtures is shown in Fig. 2. The diagram clearly indicates the existence of a significant solid solution region consisting of griseofulvin and succinic acid. Little solubility of succinic acid in griseofulvin in the solid state is evidenced. Further inspection of the phase diagram reveals that the mixture exhibits a well-defined eutectic point. The eutectic mixture has a composition of 0.71 mole of succinic acid and 0.29 mole of griseofulvin, corresponding to about 55% griseofulvin and 45% succinic acid.

The eutectic mixture consists of 2 physically separable phases. One phase is almost pure griseofulvin, while the other phase is a saturated solid solution of griseofulvin in succinic acid. The composition of the saturated solid solution is 10 moles per cent griseofulvin and 90 moles per cent succinic acid. This corresponds to about 25% griseofulvin dissolved in succinic acid. Because the ratio of griseofulvin to succinic acid at the eutectic point is thermodynamically fixed, it can be shown by simultaneous equations that the eutectic mixture is composed of 60% solid solution and 40% of almost pure griseofulvin. Since the solid solution is 25% griseofulvin, 15 parts of the 55 parts of griseofulvin found in 100 parts of eutectic mixture is in the form of a solid solution. This corresponds to 27.3% of the griseofulvin.

It has been our experience that when 2 compounds exhibit solid state interactions they frequently demonstrate interaction in aqueous solution. This phenomenon has been observed with *N*-acetyl-*p*-aminophenol and urea (4), niacinamide and ascorbic acid (9), and in sulfathiazole–urea and chloramphenicol–urea systems (10). Similarly, griseofulvin and succinic acid were found to display

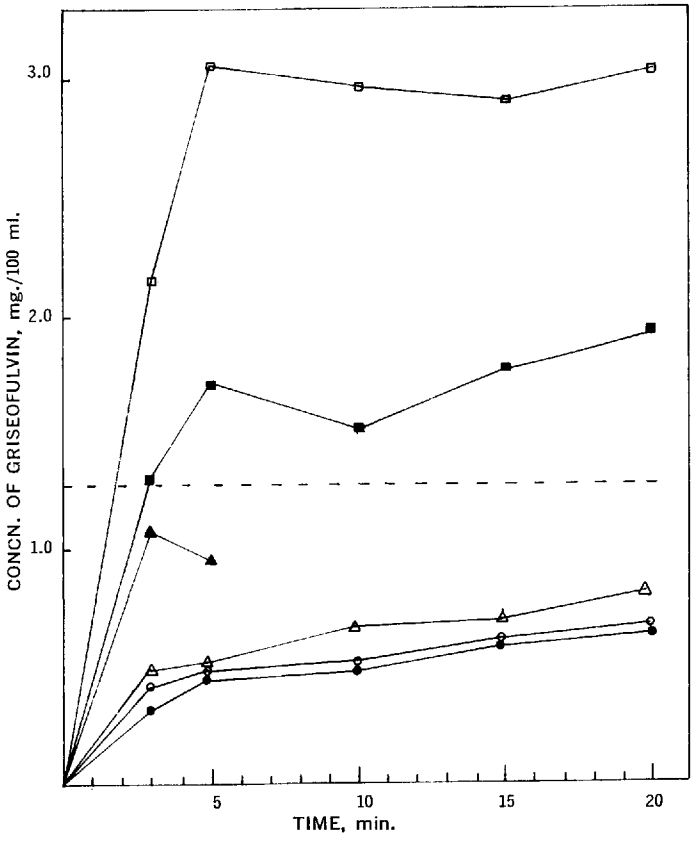


Fig. 4.—Dissolution rates of various griseofulvin and griseofulvin–succinic acid samples as determined by the oscillating bottle method. Key: ●, griseofulvin, crystalline; ▲, griseofulvin, micronized; ■, eutectic mixture; ○, physical mixture at eutectic composition; □, solid solution; △, physical mixture at solid solution composition. The dotted line indicates the equilibrium solubility of griseofulvin in water.

TABLE II.—DISSOLUTION STUDIES OF GRISEOFULVIN IN FUSED AND PHYSICAL MIXTURES WITH SUCCINIC ACID EMPLOYING THE VIAL METHOD

Sample	Form	Relative Dissolution Rate 3 min.	5 min.
Griseofulvin	Crystalline	1.0	1.0
Griseofulvin, Succinic acid	Physical mix, eutectic compn.	1.4	1.0
Griseofulvin, Succinic acid	Physical mix, solid soln. compn.	1.5	1.1
Griseofulvin	Micronized	3.5	2.1
Griseofulvin, Succinic acid	Fused, eutectic mixture	4.2	3.7
Griseofulvin, Succinic acid	Fused, solid soln.	6.9	6.7

a solution phase interaction manifested by a linear increase in the solubility of griseofulvin with increasing concentration of succinic acid. The solubility data are presented in Fig. 3.

As noted in the experimental section of this report, 2 methods were employed to evaluate the relative dissolution rates of the various samples listed in Table I. The results obtained using the oscillating vial method are shown in Fig. 4 and Table II. Table II contains relative dissolution rate data which were calculated by determining the amount of griseofulvin dissolved from a particular sample and dividing this figure by the amount of griseofulvin dissolved from the pure crystalline sample at the same time interval. At 3 min., the ratio of griseofulvin solid solution to that of the control is almost 7, indicating that the initial rate of dissolution of griseofulvin from a solid solution containing 20% griseofulvin is in the order of 7 times greater than griseofulvin alone.

The amount of dissolved griseofulvin from the solid solution at 5 min. was 2.4 times the equilibrium solubility. It is well known that reducing the particle size of a material to a very fine state of subdivision often results in supersaturation (11). Theoretically, griseofulvin is released from the solid solution in a molecular state and it is reasonable to expect such supersaturation.

It is not possible to attribute the observed increased dissolution rate to a local effect since the dissolution rate of all physical mixtures of succinic acid and griseofulvin was about the same as that of pure griseofulvin. In addition, no sample contained a sufficient amount of succinic acid to produce a bulk solubilizing effect. The increased dissolution rate of the micronized form as compared to the crystalline form of griseofulvin can be related to only one factor: decreased particle size and increased surface area. It may be concluded that since the solid solution dissolves twice as fast as the micronized form, the griseofulvin must be released in a particle size that is much smaller than the

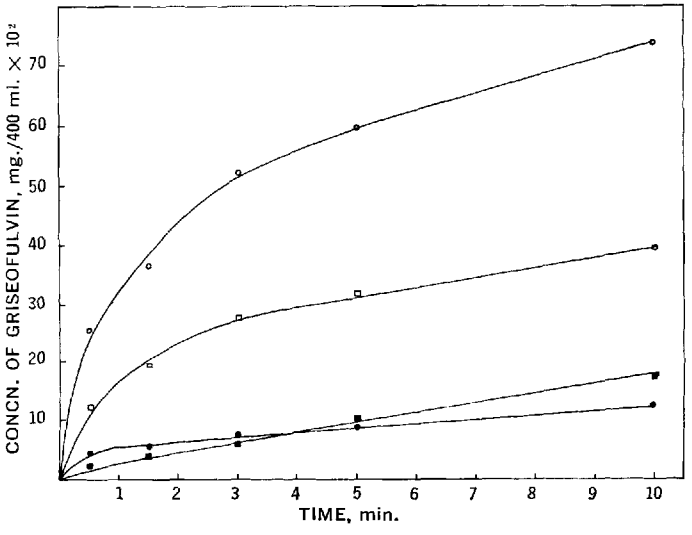
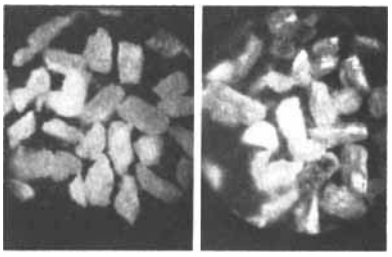


Fig. 5.—Dissolution rates of griseofulvin and griseofulvin–succinic acid samples as determined by the tape method. Key: ■, physical mixture at solid solution composition; ●, griseofulvin, crystalline; □, eutectic mixture; ○, solid solution.

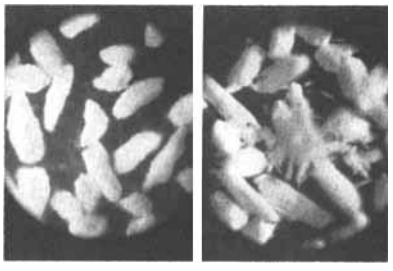
TABLE III.—DISSOLUTION STUDIES OF GRISEOFULVIN IN FUSED AND PHYSICAL MIXTURES WITH SUCCINIC ACID EMPLOYING THE TAPE METHOD

Sample	Form	Relative Dissolution Rate 0.5 min.	1.5 min.	3.0 min.	5.0 min.	10.0 min.
Griseofulvin	Crystalline	1.0	1.0	1.0	1.0	1.0
Griseofulvin, Succinic acid	Physical mix, solid soln. compn.	0.6	0.8	0.9	1.2	1.5
Griseofulvin, Succinic acid	Fused eutectic mixture	2.6	3.7	3.6	3.8	3.3
Griseofulvin, Succinic acid	Fused solid soln.	5.6	6.9	6.8	7.0	6.2



A B

Fig. 6.—Photomicrographs of crystalline griseofulvin before (*A*) and after (*B*) exposure to the dissolution medium.



A B

Fig. 7.—Photomicrographs of the griseofulvin–succinic acid eutectic mixture initially (*A*) and after 26 min. in the dissolution medium (*B*).

micronized particles theoretically approaching molecular size.

The eutectic mixture, at 3 min., dissolved 4.2 times faster than the crystalline griseofulvin, and 1.2 times faster than the micronized powder. This result is expected from the predictions of Sekiguchi *et al.* (6, 7). However, since the eutectic mixture is composed of 60% of the solid solution, it is reasonable to assume that the increased dissolution rate is attributable primarily to the presence of this solid solution in the eutectic sample. If decreased particle size does exert an effect, this effect appears inconsequential as compared to the effect of solid solution formation.

Although it may be fortuitous, the eutectic mixture which consists of 60% solid solution shows a rate at 3 min. which is just 60% that of the solid solution. Since it has been shown (4) that formation of a eutectic mixture *per se* may not increase the rate of dissolution, it is interesting to speculate that this is not just coincidental, but a physically meaningful result.

The results of the dissolution rate studies conducted with the tape method are shown in Fig. 5 and Table III. Inspection of the data indicates excellent correlation between the 2 dissolution methods. The griseofulvin from the solid solution was again found to dissolve 6–7 times faster than the pure material.

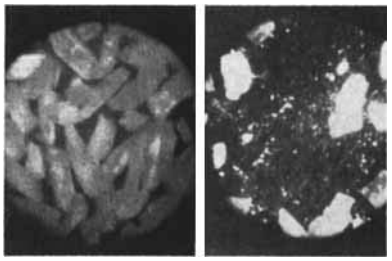
When employing the tape method in an earlier study (4), with a physical mixture of APAP and urea, a distinct local effect of the urea on the APAP was noted. In the present investigation, despite the solubilizing effect of the succinic acid on griseofulvin, no local effect was noted. The supersaturation that was found when using the vial method was absent from the data obtained with the tape method. This may be the result of differences between the 2 methods in the amount of drug excess and in the type of agitation employed.

The use of the tape method permitted the preparation of photomicrographs of the particles before and after the dissolution study. A light microscope, equipped with polarizing lenses, was employed at a magnification of 150×, in conjunction with a Polaroid Land camera.

Figure 6, *A*, shows the particles of pure griseofulvin before dissolution. Figure 6, *B*, shows the same particles after a 26-min. exposure to the dissolution medium. The only difference seems to be the disappearance of the microcrystals that appear to be associated with the griseofulvin crystals in Fig. 6, *A*.

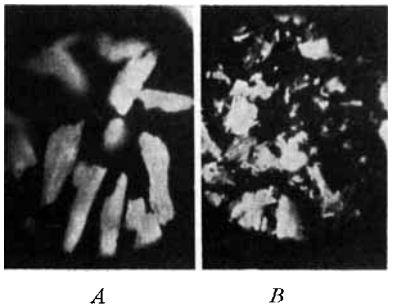
Figure 7, *A* and *B*, are photomicrographs of the griseofulvin–succinic acid eutectic mixture before and after a 26-min. dissolution study. Fragmentation of the particles is evident in Fig. 7, *B*. This is probably the result of preferential loss of the more soluble components of the individual particulates.

Figure 8, *A*, shows the particles in a physical mixture of griseofulvin and succinic acid. The striking similarity between the two crystals may be observed. After a 26-min. period in the dissolution medium (Fig. 8, *B*) only the griseofulvin crystals appeared to remain on the tape with little or no



A B

Fig. 8.—Photomicrographs of a physical mixture of griseofulvin and succinic acid present in a ratio corresponding to the solid solution composition. *A* shows both crystals; *B* shows the same area of the tape after a 26-min. exposure to the dissolution fluids; only the griseofulvin crystals remain on the tape.



A B

Fig. 9.—Photomicrographs of the griseofulvin–succinic acid solid solution before (*A*) and after (*B*) exposure to the dissolution medium.

change in size or shape. Figures 9, *A* and *B*, are photomicrographs of the solid solution containing 20% griseofulvin. Before dissolution the particles appeared somewhat more elongated and irregular than the other samples considered. After exposure to the dissolution fluids (Fig. 9, *B*) the particles seem to be sintered and greatly reduced in size.

The photomicrographs serve to support graphically the quantitative findings of the dissolution rate studies. The most rapidly soluble forms of griseofulvin, *i.e.*, the solid solution and the eutectic mixture, manifest the most dramatic alterations in their crystalline nature after exposure to the dissolution medium.

The therapeutic advantages of the griseofulvin–succinic acid solid solution are presently under investigation in our laboratories. Extrapolation of the *in vitro* findings suggests the possibility that this form will provide more rapid and complete absorption of the drug, permit a reduction in dosage, and conceivably provide a more uniform therapeutic response. Of greater significance is the fact that this investigation has demonstrated a method of physical modification which could prove more important than micronization in enhancing the absorption and therapeutic effect of many water-insoluble drugs.

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The Antitumor Agent, 1,3-Bis(2-chloroethyl)-1-nitrosourea

By TI LI LOO*, ROBERT L. DION, ROBERT L. DIXON†, and DAVID P. RALL

The new potent antitumor agent, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), is most stable at pH 4. In acid and in solutions above pH 7, it decomposes rapidly. In plasma, BCNU has a half-life of 20 min. *in vitro*, and less than 15 min. *in vivo*. Its alkylating action is not caused by the slow hydrolysis of the chlorine. BCNU is 80 per cent bound to human plasma protein at 0°. When administered intravenously to the dog, it enters the cerebrospinal fluid (CSF) readily and disappears speedily from the plasma and the CSF. The total amount of unchanged drug excreted in the urine in 4 hr. is less than 0.1 per cent of the dose. Heating at 43° for 5 hr. converts BCNU partly into 1,3-bis(2-chloroethyl)urea.

STUDIES OF the antitumor activity of derivatives of nitrosoguanidine (2) and nitrosourea (3) have led to the discovery of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), a potent cancer chemotherapeutic agent of a novel type. BCNU is remarkable in that it is highly effective not only in intraperitoneal L1210 leukemia, but also in intracerebral L1210 leukemia (4), a distinct feature seldom found in most conventional agents. Unfortunately, clinical application of BCNU is limited because of unusual delayed toxicity in animals and man (5–7).

Chemically, although considered to be an alkylating agent, BCNU differs from typical derivatives of 2-chloroethylamine in having several reaction sites in addition to the carbon–chlorine bond which are potentially liable to attack by a variety of reagents under normal physiological conditions. Besides, the resultant transient chemical species may undergo further extensive biotransformations. These interesting considerations have prompted the authors to undertake a study on some of the chemical and pharmacological properties of BCNU. The present paper summarizes the results of these studies.

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This article is dedicated to the late Dr. E. K. Marshall, Jr., Professor Emeritus of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Md. The senior author gratefully acknowledges the counsel of Dr. Marshall on numerous occasions. The present work would have been impossible without the colorimetric method for sulfanilamide of Bratton and Marshall (1).

The authors thank Dr. Thomas Johnston for a gift of 1,3-bis(2-chloroethyl)urea and 1-(2-chloroethyl)-1-nitroso-3-cyclohexylurea.

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