Table I—Initial Entrapment of Floxuridine in Liposomes

Vesicle Composition	Lipid Ratio	Percent Entrapped[a]		
		Sucrose	Floxuridine	Fluorouracil
Sphingomyelin–cholesterol–dicetyl phosphate	4.8:2.8:1.0[b]	14.3 ± 1.1[c]	11.9 ± 2.5[c]	3.6 ± 0.3[c]
Lecithin–cholesterol–dicetyl phosphate	4.8:2.8:1.0[b]	8.0	0.87	1.0
Sphingomyelin–cholesterol–dicetyl phosphate	7.0:2.0:1.0[d]	4.3[e]	3.0[e]	—
Lecithin–cholesterol–dicetyl phosphate	7.0:2.0:1.0[d]	4.1[e]	0.34[e]	—

[a] Measured after 2 min of vortexing, 2 hr of swelling at 25°, 15 min of sonication, and a second 2 hr of swelling at 25°. [b] Seventy micromoles of lipid/ml of aqueous phase. [c] Mean ± *SEM* (three determinations). [d] Fifty micromoles of lipid/ml of aqueous phase. [e] Average of two determinations.

are complex and not first order. The data are plotted on a semilogarithmic plot only as a convenient method for visualizing the relative differences observed.

There is considerable rationale for the entrapment of fluorouracil within liposomes and, as mentioned, retention has been a problem. The present results indicate that floxuridine is retained far better when sphingomyelin liposomes are used, and several other benefits, such as higher intrinsic activity against some cancers, favor the nucleoside. Preliminary results in these laboratories indicate that, after intraperitoneal administration to mice bearing L-1210 leukemia in the ascites form, the liposomes and their floxuridine content are avidly engulfed by the L-1210 cells, with large quantities of floxuridine concentrating in the cells. Further studies are in progress to ascertain the effect of such administrations on the survival times of tumor-bearing mice.

REFERENCES

(1) S. S. Cohen, J. G. Flaks, H. D. Barner, M. R. Loeb, and J. Lichtenstein, *Proc. Nat. Acad. Sci. USA*, **44**, 1004 (1958).

(2) K. L. Mukherjee and C. Heidelberger, *Cancer Res.*, **22**, 815 (1962).

(3) C. Heidelberger, L. Griesbach, O. Cruz, R. J. Schnitzer, and E. Grunberg, *Proc. Soc. Exp. Biol. Med.*, **97**, 470 (1958).

(4) C. Heidelberger, "Cancer Medicine," E. Frei and J. F. Holland, Eds., Lea & Febiger, Philadelphia, Pa., 1973, p. 776.

(5) J. J. Windheuser and J. Jato, *J. Pharm. Sci.*, **61**, 1669 (1972).

(6) G. D. Birnie, H. Kroeger, and C. Heidelberger, *Biochemistry*, **2**, 566 (1963).

(7) L. J. Lesko and R. Ganapathi, presented at the APhA Academy of Pharmaceutical Sciences, Atlanta meeting, Nov. 1975.

(8) G. Gregoriadis, *Biochem. Soc. Trans.*, **2**, 117 (1974).

(9) D. Papahadjopoulous and H. K. Kimelberg, *Prog. Surf. Sci.*, **4**, 141 (1973).

(10) E. Oldfield and D. Chapman, *FEBS Lett.*, **21**, 303 (1972).

(11) B. D. Ladbrooke and D. Chapman, *Chem. Phys. Lipids*, **3**, 304 (1969).

(12) B. E. Cohen, *J. Membr. Biol.*, **20**, 205 (1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 23, 1976, from the *Industrial and Physical Pharmacy Department, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.*

Accepted for publication August 18, 1976.

Supported by Grant 17866 from the National Cancer Institute, National Institutes of Health, Bethesda, MD 20014.

[x] To whom inquiries should be directed. Present address: Pharmacy Department, University of Connecticut Health Center, Farmington, CT 06032.

Inhibition of Catechol *O*-Methyltransferase and Transfer RNA Methyltransferases by Coralyne, Nitidine, and Related Compounds

JOHN W. LEE, JOHN O. MacFARLANE, ROBERT K. Y. ZEE-CHENG, and C. C. CHENG [x]

Abstract □ Inhibitory activity against both catechol *O*-methyltransferase and transfer RNA methyltransferases was observed among the antileukemic alkaloids coralyne, nitidine, and related synthetic alkoxy analogs. Inhibition of both classes of enzymes seems to have a similar profile. The role of water solubility of these compounds with regard to their enzyme inhibitory activity was noted.

Keyphrases □ Catechol *O*-methyltransferase—activity, effect of coralyne, nitidine, and related compounds, rat liver homogenate □ tRNA methyltransferase—activity, effect of coralyne, nitidine, and related compounds, rat liver homogenate □ Enzyme activity—catechol *O*-methyltransferase and tRNA methyltransferase, effect of coralyne, nitidine, and related compounds, rat liver homogenate □ Coralyne—effect on catechol *O*-methyltransferase and tRNA methyltransferase activity, rat liver homogenate □ Nitidine—effect on catechol *O*-methyltransferase and tRNA methyltransferase activity, rat liver homogenate □ Alkaloids, antileukemic—coralyne, nitidine, and related compounds, effect on catechol *O*-methyltransferase and tRNA methyltransferase activity, rat liver homogenate □ Structure–activity relationships—effect of coralyne, nitidine, and related compounds on catechol *O*-methyltransferase and tRNA methyltransferase activity, rat liver homogenate

Experimental evidence (1–9) has substantiated the observation of higher transfer RNA (tRNA) methyltransferase activity in fetal or malignant tumor tissue than in normal or benign tumor tissue. Also, certain chemical carcinogens increased tRNA methyltransferase activity (10, 11). These aberrant methylating enzymes may differ qualitatively from the regular tRNA methyltransferase in their specificity of action (12, 13). Certain oxygen-containing compounds possessing antileukemic activity may be tRNA 2′-*O*-methyltransferase inhibitors, and this

methylating enzyme may act *via* a complex analogous to that known for catechol *O*-methyltransferase (COMT) (14).

Therefore, preliminary enzyme inhibitory activity tests of the antileukemic alkaloids coralyne, nitidine, and related compounds (15–19) against catechol *O*-methyltransferase and tRNA methyltransferases were conducted. A number of antitumor purine nucleosides (20), the tubercidin analog of *S*-adenosylhomocysteine (21), and polyinosinate (22) have already been reported as inhibitors of tRNA (and rRNA) methyltransferases.

EXPERIMENTAL

Assay of Catechol *O*-Methyltransferase—The colorimetric assay method used was based on that reported by Herblin (23). The catechol *O*-methyltransferase enzyme extract was prepared according to the method of Axelrod and Tomchick (24) with some modification (all procedures performed at 0–5°). Male adult rat liver, 100 g, was homogenized in 400 ml of 0.15 *M* isotonic potassium chloride. The solution was centrifuged at 41,000×*g* for 30 min. The supernate was drained through cheesecloth, adjusted to pH 5.3 with 1 *N* acetic acid, and centrifuged at 800×*g* for 10 min. The precipitate was discarded, and ammonium sulfate was added to achieve 30% saturation.

This solution was again centrifuged (800×*g* for 10 min), and ammonium sulfate was added to the supernate to achieve 50% saturation. The resulting solution was centrifuged at 6600×*g* for 15 min. The precipitate was collected and dissolved in 40 ml of water and dialyzed against 0.001 *M* phosphate buffer at pH 7 for 4 hr. The enzyme preparation was then dispensed into small (1–5-ml) aliquots and frozen at −60°. The partially purified enzyme preparations used for these tests contained approximately 40–50 mg of protein/ml and 0.05–0.1 unit of enzyme/mg of protein, or approximately 0.75–1.5 units of enzyme per test.

Based upon a series of preliminary titrations and experimental studies, the following procedure was used. Reagents were added to the reaction tubes at 0–5° (ice bath) in the following quantities and order: 0.25 μmole of pH 7 tris(hydroxymethyl)aminomethane buffer, 2.5 μmoles of magnesium chloride hexahydrate, 0.5 ml of the prepared catechol *O*-methyltransferase enzyme extract, 0.1 μmole of *S*-adenosylmethionine (SAM), and 0.13 μmole of 2.68×10^{-4} *M* 4-nitrocatechol as the methyl acceptor. Distilled water was added to tubes as needed to give a final total volume of 2.5 ml.

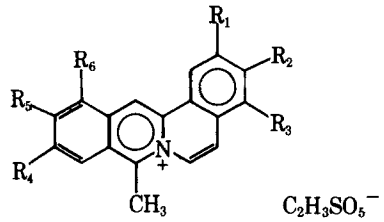
For the inhibition test, 0.5 ml of the inhibitor solution was allowed to react with 0.5 ml of the enzyme preparation at 0–5° for 5 min and then was added to the reaction mixture. This solution was allowed to remain in a 37° water bath for 45 min, followed by the addition of 2 ml of 6 *N* NaOH to terminate the enzyme reaction. Tubes containing the reaction mixture were centrifuged at 800×*g* at 5° for 10 min. The absorbance of the supernate was measured at 520 nm in a UV spectrophotometer (cell had 10-mm light path). Since at pH ≥ 9, 4-nitrocatechol exhibits a red color whereas its methylated product does not, methylation of catechol *O*-methyltransferase activity can be measured as a decrease in absorbance at 520 nm.

To determine enzyme activity, a reaction mixture was run as a control against the mixture with enzyme. The difference in the absorbance of these two mixtures was then the measure of enzyme activity. Inhibitors were generally tested at three different concentrations. The concentration of an inhibitor was calculated as the final concentration after dilution of the reaction mixture (0.5 ml in 2.5 ml or 1:5). The level of inhibition was determined by measuring the absorbance of a reaction mixture with the inhibitor and subtracting that of a reaction mixture with both the inhibitor and the enzyme.

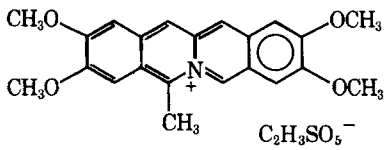
The percent of enzyme inhibition was then calculated as:

$$\% \text{ inhibition} = \frac{\Delta \text{ absorbance (reflecting enzyme activity with inhibitor)}}{\Delta \text{ absorbance (reflecting enzyme activity without inhibitor)}} \times 100 \quad \text{(Eq. 1)}$$

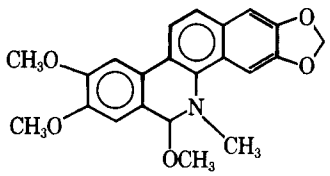
As a control of the entire procedure, a known inhibitor of catechol *O*-methyltransferase, pyrogallol, was run to achieve a "standard" curve of inhibition (100, 45, and 12% inhibition at 10^{-3}, 10^{-4}, and 10^{-5} *M*, respectively). Pyrogallol was run periodically to check that the assay was producing consistent results.



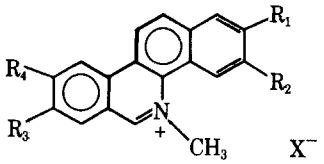
I: $R_1 = R_2 = R_4 = R_5 = OCH_3$, $R_3 = R_6 = H$
II: $R_1 = R_2 = R_5 = R_6 = OCH_3$, $R_3 = R_4 = H$
III: $R_2 = R_3 = R_4 = R_5 = OCH_3$, $R_1 = R_6 = H$



IV



VIII



V: $R_1 R_2 = OCH_2O$, $R_3 = R_4 = OCH_3$, X = Cl
VI: $R_1 R_2 = OCH_2O$, $R_3 = R_4 = OCH_3$, $X = CH_3SO_4$
VII: $R_1 R_2 = OCH_2O$, $R_3 = R_4 = OCH_3$, $X = BrCH_2CO_2$
IX: $R_1 = R_2 = R_3 = R_4 = OCH_3$, $X = SO_3F$
X: $R_1 = R_2 = R_3 = R_4 = OCOCH_3$, $X = SO_3F$
XI: $R_1 = R_2 = R_3 = R_4 = OH$, X = Cl

Assay of tRNA Methyltransferases—The activity of tRNA methyltransferases was measured by the amount of ^{14}C-methyl groups incorporated into the tRNA using the procedure of Waalkes *et al.* (25) with slight modification. The tRNA methyltransferase enzyme extraction was prepared according to the method of Kerr (26) with some modification. Male adult rat liver, 50 g, was homogenized in 200 ml of isotonic potassium chloride, and the mixture was centrifuged at 60,000×*g* for 60 min.

The supernate was drained through cheesecloth, adjusted to pH 5.3 with 1 *N* acetic acid, and centrifuged for 10 min at 6600×*g*. The precipitate was collected and dissolved in 20 ml of 0.1 *M* tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 8). This solution was then stored in small aliquots at −60° and used as the source of inhibitor-free enzyme. Since this extract contained some catechol *O*-methyltransferase activity, pyrogallol was added to the reaction mixture to suppress this activity.

The following procedure was used as the basis of a series of titrations and experimental studies. Reagents were added to the reaction tubes at 0–5° in the following order and quantities: 70 μmoles of ammonium acetate, 0.3 μmole of dithiothreitol[1], 0.3 μmole of tetrasodium ethylenediaminetetraacetate, 25 μmoles of pH 9 tris(hydroxymethyl)aminomethane hydrochloride, 0.5 μmole of magnesium chloride, 0.5 μmole of pyrogallol, enzyme extract (200 μg of protein), 100 μg of undermethylated *Escherichia coli* B tRNA[2], and 5 pmoles of ^{14}C-*S*-adenosyl-L-methionine (58 mCi/mole). The final volume of each tube was 0.5 ml.

For the inhibition test, 0.05 ml of the inhibitor solution was mixed with the enzyme extract in an ice bath (0–5°) for 5 min and then added to the reaction mixture, which was placed in a 37° water bath for 30 min. The reaction was terminated by placing the tube in an ice bath and adding 2.5 ml of 10% trichloroacetic acid. After 20 min, the reaction mixture was

[1] Calbiochem.
[2] Sigma.

Table I—Enzyme Inhibition Test Results

	Compound	COMT Inhibition, %			tRNA Methyltransferase Inhibition, %		
		10^{-3} *M*	10^{-4} *M*	10^{-5} *M*	10^{-3} *M*	10^{-4} *M*	10^{-5} *M*
I	(coralyne acetosulfate)	100	80	43	48	10	0
II	(isocoralyne acetosulfate)	(75)[a]	15	0	56	10	0
III	(neocoralyne acetosulfate)	73	35	0	64	49	33
IV	(stracoralyne acetosulfate)	—[b]	7	0	20	5	0
V	(nitidine chloride)	60	0	0	37	32	21
VI	(nitidine methosulfate)	70	0	0	46	35	11
VII	(nitidine bromoacetate)	13	10	8	58	31	5
VIII	(methoxydihydronitidine)	(100)	71	0	73	33	0
IX	(*O*-methylfagaronine fluorosulfonate)	100	34	8	100	96	18
X		100	71	0	100	94	11
XI		(74)	54	34	87	15	0
XII		6	2	0	0	0	0
XIII		6	3	0	18	2	0
XIV		0	0	0	15	0	0

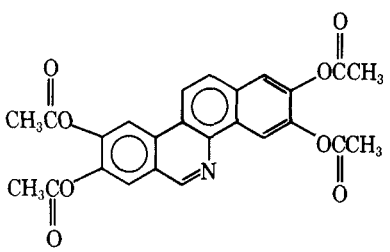
[a] Values in parentheses denote those obtained by graph extrapolation. [b] Insoluble.

filtered through a type HA individual filter (0.45-μm pore size) and washed with 20 ml of 5% trichloroacetic acid. The residue was then air dried and placed into vials with 10 ml of a mixture of 91% of 2,5-diphenyloxazole and 9% of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene. Radioactivity was measured in a liquid scintillation counter.

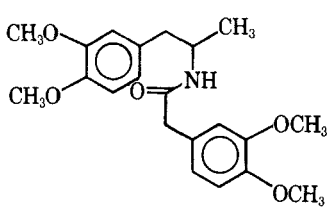
To determine enzyme activity, a reaction mixture without tRNA was run as a control against a reaction mixture with tRNA. The difference in ^{14}C-incorporation, measured in counts per minute between these two mixtures, is a measure of enzyme activity (*A*).

Inhibitors were generally tested at three different concentrations. The concentration of an inhibitor was calculated as the final concentration after dilution of the reaction mixture. The level of inhibition was determined by subtracting the counts per minute of a reaction mixture with the inhibitor only from that of a reaction mixture with both the inhibitor and the enzyme (*B*). The percent of inhibition was then calculated as $[(A - B)/A] \times 100$.

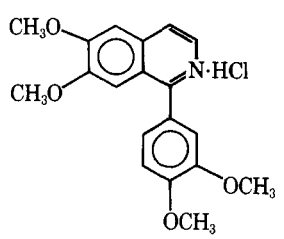
An ideal standard for inhibition of tRNA methyltransferases is yet to be found. Nicotinamide (27, 28) and ascorbic acid (40 and 51% inhibition, respectively, at 10^{-3} *M*) were used as controls throughout the tRNA methyltransferase inhibitory tests.



XII



XIII



XIV

RESULTS AND DISCUSSION

Preliminary enzyme inhibitory results are given in Table I. The first nine compounds (I–IX) were active against leukemias P-388 and L-1210 (15–19), whereas the last five compounds (X–XIV) were inactive. Although no definite conclusion should be drawn at this stage, the results suggest that there is a parallel correlation between the antileukemic activity of these alkoxy compounds and their inhibitory activity against both classes of enzyme. In addition, inhibition against both enzymes seems to have a similar profile.

Solubility characteristics of these compounds in water play an important role in enzyme inhibition. Compound IV (stracoralyne acetosulfate), for example, is quite insoluble in water. Thus, the observed activity may not reflect the true value.

Although X showed high inhibitory activity against both enzymes, it was without effect against leukemias P-388 and L-1210. This finding is probably due to the relative ease of hydrolysis of the acetoxy groups *in vivo;* the resulting phenolic compounds may not be readily transported to the proper biological site to achieve the desired antineoplastic action. (The fact that the tetrahydroxy compound, XI, possessed enzyme inhibitory activity but was inactive against leukemias P-388 and L-1210 further substantiates the proposition.) The corresponding uncharged compound, XII, which is much less soluble in water, was inactive against both enzyme and leukemia systems.

Since the presently studied tRNA methyltransferases are composed of a mixture of methylating enzymes and since purified enzymes such as tRNA *O*-methyltransferase and tRNA *N*-methyltransferase could not be realized, a definite relationship could not be drawn as to whether the inhibitory action against tRNA methylating enzymes is directed to any specific portion of the macromolecule. The original tRNA 2′-*O*-methylation hypothesis (14) thus remains to be examined. Therefore, single tRNA methyltransferases that specifically methylate oxygen, nitrogen, or other atoms should be isolated and purified before further study.

REFERENCES

(1) S. J. Kerr, *J. Biol. Chem.*, **247,** 4248 (1972).

(2) S. E. Wright, R. E. Gallagher, R. C. Ting, and R. C. Gallo, *Cancer Res.*, **33,** 2513 (1973).

(3) B. Sheid, T. Lu, and J. H. Nelson, Jr., *ibid.*, **33,** 2518 (1973).

(4) T. P. Waalkes, S. R. Dinsmore, and J. E. Mrochek, *J. Natl. Cancer Inst.*, **51,** 271 (1973).

(5) B. Sheid, T. Lu, and J. H. Nelson, Jr., *Cancer Res.*, **34,** 2416 (1974).

(6) N. P. Trifunac and A. I. Krasna, *Biochemistry,* **13,** 2403 (1974).

(7) M. Hayashi and A. C. Griffin, *Cancer Res.*, **34,** 3311 (1974).

(8) J. E. Heady and S. J. Kerr, *ibid.*, **35,** 640 (1975).

(9) R. Gantt, G. H. Smith, and B. T. Julian, *ibid.*, **35,** 1847 (1975).

(10) R. L. Hancock, *Biochim. Biophys. Acta,* **281,** 472 (1972).

(11) R. L. Hancock and P. I. Forrester, *Cancer Res.*, **33,** 1747 (1973).

(12) F. Nau, F. Garbit, and J.-M. Dubert, *Biochim. Biophys. Acta,* **277,** 80 (1972).

(13) M. C. Liau, C. M. O'Rourke, and R. B. Hurlbert, *Biochemistry,* **111,** 629 (1972).

(14) C. C. Cheng, *J. Pharm. Sci.*, **61**, 645 (1972).
(15) K. Y. Zee-Cheng and C. C. Cheng, *ibid.*, **61**, 969 (1972).
(16) K. Y. Zee-Cheng and C. C. Cheng, *J. Heterocycl. Chem.*, **10**, 85, 867 (1973).
(17) K. Y. Zee-Cheng, K. D. Paull, and C. C. Cheng, *J. Med. Chem.*, **17**, 347 (1974).
(18) R. K. Y. Zee-Cheng and C. C. Cheng, *ibid.*, **18**, 66 (1975).
(19) *Ibid.*, **19**, 882 (1976).
(20) E. Wainfan and B. Landsberg, *Biochem. Pharmacol.*, **22**, 493 (1973).
(21) J. K. Coward, D. O. Bussolotti, and C.-D. Chang, *J. Med. Chem.*, **17**, 1286 (1974).
(22) M. C. Liau, J. B. Hunt, D. W. Smith, and R. B. Hurlbert, *Cancer Res.*, **33**, 323 (1973).
(23) W. F. Herblin, *Anal. Biochem.*, **51**, 19 (1973).
(24) J. Axelrod and R. Tomchick, *J. Biol. Chem.*, **233**, 702 (1958).
(25) T. P. Waalkes,.R. H. Adamson, D. Riddick, and R. C. Gallo, *Arch. Int. Pharmacodyn. Ther.*, **196**, 345 (1972).
(26) S. J. Kerr, *Biochemistry*, **9**, 690 (1970).
(27) R. M. Halpern, S. Q. Chaney, B. C. Halpern, and R. A. Smith, *Biochem. Biophys. Res. Commun.*, **42**, 602 (1971).
(28) L. Buch, D. Streeter, R. M. Halpern, L. N. Simon, M. G. Stout, and R. A. Smith, *Biochemistry*, **11**, 393 (1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 14, 1976, from the *Midwest Research Institute, Kansas City, MO 64110.*

Accepted for publication September 15, 1976.

Supported by Contract N01-CM-33743 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

The authors thank Dr. Harry B. Wood, Jr., for interest and encouragement.

[x] To whom inquiries should be directed.

Pharmaceutical Applications of Solid Dispersion Systems: X-Ray Diffraction and Aqueous Solubility Studies on Griseofulvin–Polyethylene Glycol 6000 Systems

WIN L. CHIOU

Abstract □ The X-ray diffraction method was used to characterize physicochemical properties of griseofulvin dispersed in polyethylene glycol 4000 and 6000. Results indicate a negligible or very limited solid solubility of griseofulvin in the pulverized solid dispersions. Pulverization and aging had pronounced effects on the X-ray diffraction spectrum. Results from aqueous solubility studies of griseofulvin in various concentrations of polyethylene glycol 6000 further indicate weak or insignificant interactions between the drug and the carrier. Mechanisms are postulated to account for the reported marked enhancement of dissolution rates and oral absorption of griseofulvin dispersed in these carriers.

Keyphrases □ Griseofulvin—dispersed in polyethylene glycol 6000, X-ray diffraction study, aqueous solubility, effects of pulverization and aging □ Polyethylene glycol 6000—dispersions with griseofulvin, X-ray diffraction study, aqueous solubility, effects of pulverization and aging □ Solid dispersions—griseofulvin–polyethylene glycol 6000, X-ray diffraction study, aqueous solubility, effects of pulverization and aging □ Dispersions, solid—griseofulvin–polyethylene glycol 6000, X-ray diffraction study, aqueous solubility, effects of pulverization and aging □ Solubility, aqueous—griseofulvin–polyethylene glycol 6000 dispersions, X-ray diffraction study □ X-ray diffraction—study, griseofulvin–polyethylene glycol 6000 dispersion, effects of pulverization and aging □ Antifungal agents—griseofulvin dispersed in polyethylene glycol 6000, X-ray diffraction study, aqueous solubility

The *in vitro* dissolution and oral absorption of griseofulvin in dogs and humans were markedly enhanced by the formation of solid dispersions in polyethylene glycol 6000 (1–3). Recently, these results were confirmed with a newly introduced commercial product[1] in single- (4) and multiple- (5) dose studies using larger numbers of test subjects. The new product requires only half of the dose recommended for conventional micronized products. Furthermore, the incidence of side effects decreased with this new formulation (5).

Although it was proposed that the enhancement of dissolution and absorption of griseofulvin dispersed in the polymer was primarily due to the molecular and colloidal dispersion of the drug in the highly water-soluble carrier (1–3), no extensive experimental data regarding physicochemical properties of such a dispersed system have been reported.

This paper reports the detailed results of X-ray diffraction and aqueous solubility studies on such a system. The X-ray diffraction data do not support the previous postulate regarding the extensive formation of a solid solution of griseofulvin in polyethylene glycol polymers. The aqueous solubility study was performed to investigate the possible interaction between the drug and the polymer in an aqueous medium. Findings from the present study should be valuable to the development of solid dispersion dosage forms of other poorly water-soluble drugs utilizing the same type of carriers. Some preliminary results were discussed in a review article (6).

Although different average molecular weights of polyethylene glycol polymers can be used as dispersion carriers (1–3), only polymer 6000 was studied in detail in the present investigation. This polymer has much less absorption in the UV and visible region than polymer 20,000 and thus interferes less with the direct spectrophotometric analysis of drugs (1). Furthermore, based on the author's experience, the solid dispersions prepared with this polymer were generally much easier to pulverize than those prepared with other molecular weights of the polymer.

[1] Gris-PEG, Dorsey Laboratories, Lincoln, Neb.