Quantitative Evaluation of Topically Applied Pilocarpine in the Precorneal Area

AVINASH G. THOMBRE * and KENNETH J. HIMMELSTEIN *‡x

Received July 26, 1982, from the **Department of Chemical and Petroleum Engineering, University of Kansas, Lawrence, KS 66045.* Accepted for publication January 10, 1983. ‡Present address: Inter$_x$ Research Corporation, Merck Sharp & Dohme Research Laboratories, Lawrence, KS 66044.

Abstract □ The bioavailability of topically applied pilocarpine is poor due to various loss mechanisms that serve to lessen delivery of the drug to the aqueous humor. Precorneal drainage and other routes of loss have been investigated qualitatively as well as quantitatively. The mechanistic models proposed to date suffer from the drawback of being dependent on our understanding of the mechanism of drug transport through the corneal membrane. This report quantifies the initial disposition of topically applied drugs and their availability for systemic and local absorption. The rate constant for the conjunctival absorption is determined and is independent of the mechanism of transcorneal transport.

Keyphrases □ Pilocarpine—quantitative evaluation, topically applied in the precorneal area, bioavailability, disposition □ Bioavailability—quantitative evaluation of topically applied pilocarpine in the precorneal area, disposition □ Disposition, precorneal—quantitative evaluation of topically applied pilocarpine, bioavailability

The precorneal disposition of pilocarpine greatly influences its bioavailability. The factors that affect the concentration of a topically applied drug solution in the precorneal area are shown schematically in Fig. 1.

When a drug is instilled into the eye, a large loss occurs *via* drainage. The normal resident tear volume in rabbits is reported to be 7.5 μL (1), only slightly larger than that in humans. The normal dropper used commercially in ophthalmic drug solutions delivers ~40–50 μL. Since the cul-de-sac of the eye cannot hold such a large volume of liquid, a substantial portion of the drop is lost due to overflow. The remainder is immediately subjected to a physiological drainage mechanism. This drainage is proportional to the excess volume and continues until the total tear volume is restored to the normal lacrimal volume in ~5 min, independent of the drop size (1). Another route of drug loss in the precorneal area is the normal tear turnover which is ~15–16%/min in humans (2, 3) and ~9%/min in rabbits (1). Tear turnover serves to dilute the drug and hence, reduce the gradient for transport through the cornea. An additional, associated problem is that of induced lacrimation. For reasons of stability, most marketed pilocarpine solutions are at a pH of 4–5. The physiological pH of the tear fluid is much higher, ~7.47 (4). Since the average dose size is much greater than the volume of the tears, the drug solution overwhelms the buffering capacity of the tear film, resulting in an increased production of tears.

Other factors influencing the precorneal disposition of pilocarpine include drug–protein interactions (5). Although the protein binding is reversible, the tear turnover removes both free as well as bound drug. A factor that enhances bioavailability to some extent is the evaporation of tears. The rate of evaporation from the eye of the rabbit is 2.8 μL/h/cm^2 (6). Evaporation of tears serves to increase the concentration of the drug in the tear film and, hence, increase the gradient for productive absorption.

A precorneal factor of great importance with regard to the toxicological effects of topically applied drugs is the loss through the conjunctiva. The conjunctival area is ~6 times larger than the corneal area and is highly vascularized. Therefore, the conjunctiva can be a major route for entry of the drug into systemic circulation.

A mechanistic representation of the precorneal area, presented by Himmelstein *et al.* (7), took into account the volume-dependent drainage and the effect of tear turnover based on the work of Chrai *et al.* (1). The corneal transport of the drug was assumed to be *via* a linear diffusional first-order clearance parameter. Although conjunctival absorption was not considered, a framework was established for a mechanistic understanding of the drug disposition from topical instillation to reaching the aqueous humor. The model proposed by Makoid and Robinson (8) used a first-order elimination term to describe loss from the precorneal area. The major disadvantage of their model is that it does not consider volume-dependent drainage from the precorneal area; the cornea was assumed to be a homogeneous membrane. Lee and Robinson (9) extensively describe pilocarpine disposition in the precorneal area. They considered the corneal epithelium as a separate drug-containing tissue and included the stroma and the endothelium with the aqueous humor. This was done to incorporate the findings of Seig and Robinson (10) that the cornea acts as both a barrier as well as a depot for the drug. Miller (11) proposed a pharmacokinetic model to describe the fate of pilocarpine in the internal eye. The precorneal area in the model was not treated mechanistically. The concentration of pilocarpine in the tear film was measured experimentally and used as a forcing function for corneal transport. Mishima (12) has reviewed the literature on the topical administration of ocular drugs. The concentration of the drug in the tear film was assumed to decrease exponentially with time and the corneal uptake was assumed to be governed by either a first-order mass transfer coefficient or by partitioning of the drug into the epithelial barrier.

Due to the complex nature of the corneal membrane, a mechanistic understanding of the transport of ocular drugs through the cornea is severely limited. The *in vitro* ex-

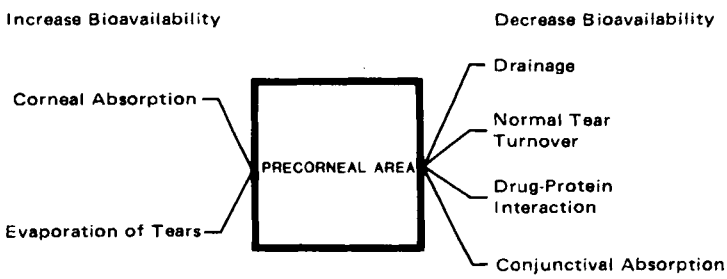

Figure 1—*Precorneal factors that influence bioavailability of topically applied ophthalmic drugs.*

0022-3549/84/0200-0219$01.00/0

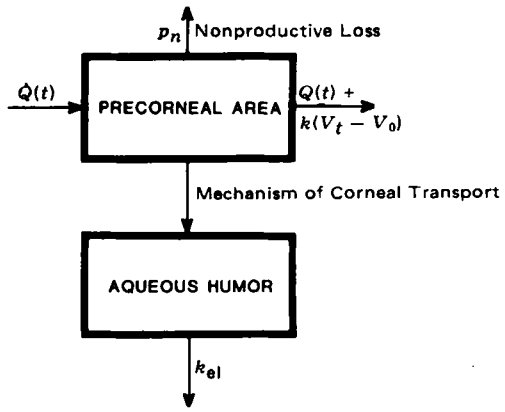

Figure 2—*Schematic diagram of the proposed model for drug disposition in the precorneal area.*

perimental data obtained by Mosher and Mikkelson (13) for the corneal transport of the *n*-alkyl *p*-aminobenzoates indicate that corneal transport is by diffusion through a trilaminate consisting of the epithelium, stroma, and the endothelium. Since the epithelium and the endothelium are more lipid in nature than the stroma, the partitioning and solubility of the drug in the individual laminates are important factors governing the transport. However, due to experimental difficulties, the individual resistances of the corneal membranes have not been estimated. In the pharmacokinetic models proposed to date, various simplifying assumptions have been made concerning the transport through the cornea. The rate constant describing the loss of drug through the conjunctiva is thus a function of these assumptions.

Here, a detailed model for the precorneal area is presented without making any assumptions about the mechanism of corneal transport. On the basis of the model, disposition of pilocarpine due to various factors are estimated quantitatively.

THEORETICAL

The proposed model for the precorneal area is shown schematically in Fig. 2. It can be considered as an extension of the model proposed by Himmelstein *et al.* (7) and Lee and Robinson (9), taking into account the volume-dependent drainage loss. The nonproductive loss is described by a first-order rate constant, p_n, and is primarily the conjunctival absorption. The analysis of the proposed model differs from previously reported analysis in the estimation of the corneal drug uptake, which is determined directly from experimentally reported pilocarpine levels in the aqueous humor (14).

The differential equation governing the concentrations of pilocarpine in the precorneal area is developed in a manner analogous to that of Himmelstein *et al.* (7). A mass balance for the amount of tear fluid in the precorneal area (Eq. 1) is made assuming that the solution has the density of water and that the nonproductive and productive loss of pilocarpine do not significantly affect the tear fluid volume:

$$\text{Rate of change in fluid volume} = \text{Rate of flow in} - \text{Rate of flow out} \quad \text{(Eq. 1)}$$

$$\frac{dV_t}{dt} = Q_t - [Q_t + K(V_t - V_0)] \quad \text{(Eq. 2)}$$

where V_t is the total volume in the precorneal area at any given time; V_0 is the normal resident tear volume; Q_t is the normal tear production rate; and K is a proportionality constant that is a function of the instilled drop size, V_d. Equation 2 has been solved analytically (1, 7) subject to the initial condition, $V_t = V_d + V_0$ at $t = 0$:

$$V_t = V_d \exp(-Kt) + V_0 \quad \text{(Eq. 3)}$$

Table I—Concentration of Pilocarpine in the Precorneal Area Following Topical Instillation of 25 μL of 1.00×10^{-2} M Pilocarpine Nitrate[a]

Minutes	Pilocarpine Concentration in Tears, μg/mL[b]
1	1690 (120)[c]
2	830 (220)
3	330 (90)
4	170 (40)
5	82 (26)

[a] Taken from Ref. 6. [b] Concentrations are based on pilocarpine alkaloid and are the average of seven determinations. Numbers in parentheses are the *SEM*.

The mass balance for pilocarpine in the tear fluid is given by:

$$\text{Rate of change of mass in tear fluid} = \text{Rate of transfer in by flow} - \text{Rate of loss by drainage} - \text{Rate of nonproductive loss} - \text{Rate of productive loss} \quad \text{(Eq. 4)}$$

The productive loss from the precorneal area is the drug transported to the aqueous humor. The rate of this productive loss is estimated as follows. Figure 3 shows the experimentally obtained levels of pilocarpine (14) in the aqueous humor after topical administration of 25 μL of a 1.00×10^{-2} M pilocarpine solution in the rabbit eye. It is seen from the figure that, for the first 5 min after instillation of the drug, the concentration in the aqueous humor increases approximately linearly with time. Thus, for the initial 5 min, the rate of productive loss is a constant. It should be noted that only the first 5 min postinstillation are critical with regard to defining the concentration gradient for drug between the precorneal area and the cornea. Table I shows the concentrations of pilocarpine in the tear film determined experimentally by Miller (6). It can be seen that the drug concentration drops to <4% of its initial value in 5 min. The initial concentration for a 25-μL dose of 1.00×10^{-2} M pilocarpine is 2083.5 μg/mL. Seig and Robinson (10) have also reported that rinsing the precorneal area 5 min after dosing has no effect on the ocular bioavailability of topically applied pilocarpine.

From Fig. 3, the slope of the aqueous humor concentration profile, assuming it to be linear for the first 5 min, was calculated to be 0.0960 μg/mL·min. Since the volume of the aqueous humor is 0.311 mL, the rate of loss of drug into the aqueous humor or the rate of productive loss in Eq. 4 is calculated to be 0.0299 μg/min. The assumption that the aqueous humor concentration profile is linear over the first 5 min is arbitrary and is justified on an empirical basis. In fact, this study attempts to obtain the precorneal factors without making any assumptions about the nature of transcorneal transport. Equation 4 can now be written as:

$$\frac{dV_tC_t}{dt} = 0 - [Q_t + k(V_t - V_0)]C_t - p_nC_t - 0.0299 \quad \text{(Eq. 5)}$$

By taking the indicated derivative and substituting the value of V_t from Eq. 3 into this expression, a simplified relationship is obtained:

$$\frac{dC_t}{dt} = \frac{-Q_tC_t - p_nC_t - 0.0299}{V_d \exp(-Kt) + V_0} \quad \text{(Eq. 6)}$$

Equation 6 can be solved analytically as:

$$-\frac{1}{(Q_t + p_n)} \ln\left(C_t + \frac{0.0299}{(Q_t + p_n)}\right) = \frac{t}{V_0} + \frac{1}{V_0K} \ln[V_0 + V_d \exp(-Kt)] + C \quad \text{(Eq. 7)}$$

where C is the constant of integration to be determined from the initial condition, *viz.*, $C_t = 2083.5$ μg/mL at $t = 0$.

Table II—Parameters of the Proposed Model

Parameter	Value	Reference
V_0	7.5 μL	1
V_d	25 μL	Experimental
V_{ah}[a]	311 μL	7
Q_t	0.66 μL/min	7
K	$(0.25 + 0.0113\ V_d)\ \text{min}^{-1}$	1
p_n	(estimated in terms of the model)	

[a] Volume in the aqueous humor.

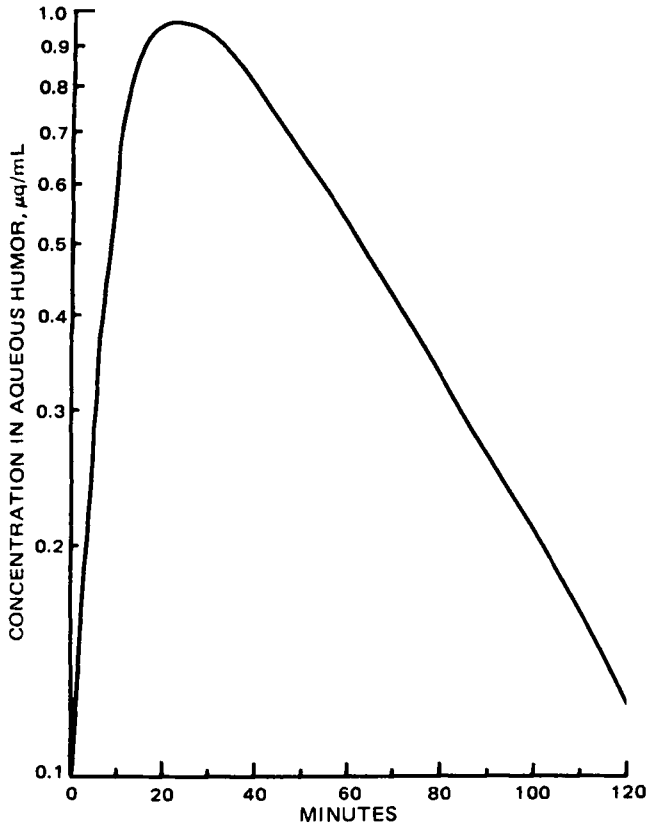

Figure 3—*Experimental Pilocarpine Concentrations in the Aqueous Humor (pH 6.24). Taken from Ref. 12.*

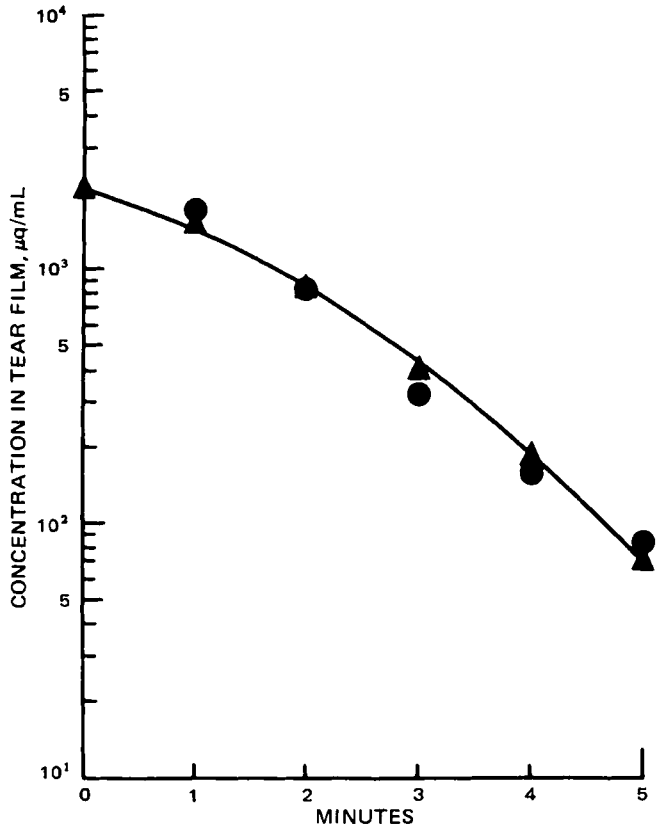

Figure 4—*Experimental [Lee and Robinson, 1979 (●); Miller, 1980 (▲)] and theoretical concentrations, according to the model (—), of pilocarpine in the tear film.*

RESULTS AND DISCUSSION

Except for the rate constant for nonproductive loss, p_n, all other parameters of the present model are known from experimental measurements. The value of K is taken as that obtained by Chrai *et al.* (1); the values used and their sources are given in Table II. The first-order rate constant for the nonproductive absorption, p_n, was evaluated in terms of the present model. Since Eq. 6 describing the concentration profile of pilocarpine in the precorneal area is nonlinear, the method of Pattern Search (15) was used to determine p_n. The objective function was formulated as:

$$J = \sum_{\text{all data}} (C_{t,\text{exp}} - C_{t,\text{model}})^2 \qquad \text{(Eq. 8)}$$

The experimental data obtained by Miller (6), presented in Table I, was used for $C_{t,\text{exp}}$. Since the concentration of pilocarpine in the precorneal area at $t = 0$ was used as the initial condition to numerically solve Eq. 6, the precorneal concentration profile is forced to pass through the point (0.0,2083.5). The value of p_n for which the objective function is minimum was found to be 8.84 μL/min.

The model-generated tear film pilocarpine concentration profile is plotted in Fig. 4. For comparison, the profiles obtained experimentally by Miller (6) and Lee and Robinson (9) have also been plotted in Fig. 4. Lee and Robinson simulated their profile with a value of 8.38 μL/min for the nonproductive loss parameter. It can be seen that the model predicts the experimental data very well. The slight curvature in the tear film pilocarpine concentration profile is also accounted for by the proposed model. This curvature indicates that the decline of pilocarpine concentration in the tear film is not a first-order process.

At this point, a distinction is made concerning the amount of pilocarpine in the precorneal area and the concentration of pilocarpine in the tear film. Evaluating the precorneal loss factors with respect to amount as well as concentration of the drug is important, since the concentration of the drug in the precorneal area determines the driving force for the corneal absorption and the amount of drug lost to the conjunctiva determines the amount that is available for systemic absorption leading to undesirable toxicological effects of the drug.

The relative amounts of loss of pilocarpine to the various loss mechanisms considered in the present model can be obtained by integration of each of the terms in the right-hand side of Eq. 4. This integration was done numerically using the composite Simpsons' rule (Table III). The above analysis shows that for 25 μL of a 0.01 M dose instilled into the eye, 52.2% is absorbed by the conjunctiva. It should be noted from Table III that up to 1 min postinstillation, the loss of pilocarpine *via* drainage is comparable with the loss due to conjunctival absorption. However, after 1 min postinstillation, the nonproductive loss is greater than that due to drainage. As mentioned earlier, drug absorbed by the conjunctiva gets into systemic circulation.

The contributions of the various precorneal factors to the decrease in the concentration of pilocarpine in the tear film is evaluated similarly by integrating the components on the right-hand side of Eq. 6 (Table IV). It is seen that the conjunctival absorption contributes 92.57% of the total decrease in pilocarpine concentration at the end of the first 5 min post-

Table III—Precorneal Disposition of Pilocarpine Following the Topical Instillation of 25 μL of 1.00×10^{-2} M Pilocarpine Nitrate

Minutes	Productive Loss	Loss Due to Tear Turnover, μg	Nonproductive Loss	Drainage Loss	Amount in Precorneal Area
0	0	0	0	0	67.7144
1	0.0299	1.1689	15.6574	18.5671	32.2911
2	0.0598	.9322	25.8825	25.7324	14.1074
3	0.0897	2.3579	31.5846	28.0953	5.5868
4	0.1196	2.5589	34.2767	28.7550	2.0042
5	0.1495	2.6398	35.3607	28.9120	0.6524

Table IV—Contributions of Precorneal Factors in the Decrease of Pilocarpine Concentration in the Tear Film Following Topical Instillation of 25 μL of 1.00×10^{-2} M Pilocarpine Nitrate

Minutes	Productive Loss	Loss Due to Tear Turnover, μg/mL	Nonproductive Loss	Concentration in Precorneal Area
0	0	0	0	2083.5198
1	1.1258	43.5164	582.9035	1455.9741
2	2.7230	83.7475	1121.8004	875.2490
3	4.8423	113.5022	1520.3654	444.8103
4	7.4670	130.9236	1753.7251	191.4049
5	10.5208	139.1052	1863.3178	70.5768

instillation. Thus, it should be noted that although drainage is responsible for 42.7% of the total amount of drug instilled, it is the conjunctival absorption that is primarily responsible for the dramatic fall in the tear film pilocarpine concentration. The tear turnover is next in importance, contributing 6.9% of the decrease of concentration. Due to the rapid decline of drug concentration in the tear film, there is considerable loss of the driving force for productive drug absorption resulting in the poor bioavailability of topically administered drugs. Thus, decreasing the nonproductive loss of drug through the conjunctiva is as important a consideration as is circumventing the drainage loss in attempts to increase the bioavailability.

CONCLUSIONS

A model has been presented to account for the precorneal disposition of topically applied pilocarpine solutions. Due to the unknown nature of transcorneal penetration, the precorneal model was solved without making any assumptions about the mechanism of corneal transport. Experimentally obtained aqueous humor pilocarpine concentrations were used to obtain the rate of productive absorption. The rate constant for nonproductive loss, p_n, was found to be 8.84 μL/min, comparable with that used by Lee and Robinson (9).

Analysis of the model leads to a clearer understanding of the relative importance of the precorneal factors in reducing both the amount of drug in the precorneal area and the concentration of the drug in the tear film. It was shown that the conjunctival absorption is an important factor in reducing the amount of drug in the precorneal area and is the single-most important factor responsible for the reduction of drug concentration in the tear film and, hence, affects the driving force for productive absorption.

REFERENCES

(1) S. S. Chrai, T. F. Patton, A. Mehta, and J. R. Robinson, *J. Pharm. Sci.*, **62**, 1112 (1973).

(2) S. Mishima, A. Gasset, S. D. Klyce, and J. L. Baum, *Invest. Ophthalmol.*, **5**, 264 (1966).

(3) M. J. Puffer, R. W. Nealt, and R. F. Brubaker, *Am. J. Ophthalmol.*, **89**, 369 (1980).

(4) A. Longwell, S. Birss, N. Keller, and D. Moore, *J. Pharm. Sci.*, **65**, 1654 (1976).

(5) T. J. Mikkelson, S. S. Chrai, and J. R. Robinson, *J. Pharm. Sci.*, **62**, 1648 (1973).

(6) S. C. Miller, Ph.D. Dissertation, University of Kansas (1980).

(7) K. J. Himmelstein, I. Guvenir, and T. F. Patton, *J. Pharm. Sci.*, **67**, 603 (1978).

(8) M. C. Makoid and J. R. Robinson, *J. Pharm. Sci.*, **68**, 435 (1979).

(9) V. H. Lee and J. R. Robinson, *J. Pharm. Sci.*, **68**, 673 (1979).

(10) J. W. Seig and J. R. Robinson, *J. Pharm. Sci.*, **65**, 1816 (1976).

(11) S. C. Miller, K. J. Himmelstein, and T. F. Patton, *J. Pharmaceut. Biopharm.*, **9**, 653 (1981).

(12) S. Mishima, *Invest. Ophthalmol. Visual Sci.*, **21**, 504 (1981).

(13) G. L. Mosher and T. J. Mikkelson, *Int. J. Pharmaceut.*, **2**, 239 (1979).

(14) T. F. Patton and J. R. Robinson, *J. Pharm. Sci.*, **65**, 1295 (1976).

(15) R. L. Fox, in "Optimization Methods for Engineering Design," Addison Wesley, 1971.

Antineoplastic Activity of Tetrakis-μ-(trimethylamine-boranecarboxylato)-*bis*(trimethylamine-carboxyborane)dicopper(II) in Ehrlich Ascites Carcinoma

I. H. HALL *, B. F. SPIELVOGEL ‡, and A. T. McPHAIL ‡

Received June 24, 1982, from the **Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514* and the ‡*Paul M. Gross Chemical Laboratory, Duke University, Durham, NC 27706.* Accepted for publication December 12, 1982.

Abstract □ A binuclear copper(II) complex derived from trimethylamine carboxyborane [tetrakis-μ-(trimethylamine-boranecarboxylato)-*bis*-(trimethylamine-carboxyborane)dicopper(II)] was shown to have antineoplastic activity in the Ehrlich ascites carcinoma screen. Metabolic studies demonstrated that the compound suppressed DNA and protein syntheses. The inhibition of DNA synthesis appeared to be due to reduction of the DNA polymerase activity and the regulatory enzymes of *de novo* purine synthesis. Preliminary data suggest that the compound is an initiation inhibitor of protein synthesis in Ehrlich ascites cells.

Keyphrases □ Copper(II) complexes—with trimethylamine carboxyborane, antineoplastic activity, Ehrlich ascites carcinoma screen □ Trimethylamine carboxyborane—complex with copper(II), antineoplastic activity, Ehrlich ascites carcinoma screen □ Antineoplastic agents—potential, copper(II)–trimethylamine carboxyborane complex, Ehrlich ascites carcinoma screen

Previously, series of trimethylamine cyanoborane and trimethylamine carboxyborane derivatives were reported to be active as antineoplastic agents against Ehrlich ascites carcinoma, Walker 256 carcino-sarcoma, P388 lymphocytic leukemia, B16 melanoma, and Lewis lung carcinoma growth (1, 2). Trimethylamine cyanoborane in Ehrlich ascites cells effectively inhibited DNA and protein syntheses as well as DNA polymerase and thymidylate synthetase activities. Scheller *et al.* (3) have demonstrated that amine carboxyboranes in dilute solutions bind to metal ions such as Zn^{2+} and Cu^{2+} as simple carboxylates and not as chelates. The synthesis and antineoplastic ac-

0022-3549/84/0200-0222$01.00/0