**Table V—Apparent Absorption Rate of Procainamide in Normal Volunteers**

| Subject | $K_a$, Capsule, $min^{-1}$ | $K_a$, Sustained Release, $min^{-1}$ | $k_0$, Intravenous Infusion, mg/min |
|---|---|---|---|
| S.R. | 0.0045 | 0.0034 | 29.3 |
| C.H. | 0.0077 | 0.0046 | 37.6 |
| B.M. | 0.0107 | 0.0058 | 33.1 |
| F.N. | 0.0092 | 0.0045 | 29.9 |
| P.Gt. | 0.0198 | 0.0040 | 42.0 |
| C.F. | 0.0173 | 0.0036 | 31.1 |
| G.B. | 0.0116 | 0.0022 | 60.1[a] |
| P.R. | 0.0866 | 0.0027 | 28.6 |
| T.C. | 0.0693 | 0.0051 | 30.8 |
| P.Ga. | 0.0866 | 0.0026 | 42 |
| L.D. | 0.0462 | 0.0041 | 27.0 |
| Mean | 0.0336 | 0.0039 | 33.1 |
| *SEM* | 0.0098 | 0.0003 | 1.7 |

[a] This value was not used in the calculation of the mean $k_0$ since when it was deleted it fell more than 5 *SD* above the mean.

## CONCLUSIONS

1. It seems to be possible to delay the *in vivo* absorption rate of procainamide in humans at least an order of magnitude and still achieve satisfactory bioavailability.
2. The method of Loo and Riegelman seems to be satisfactory for the estimation of absorption rate constants for procainamide in humans.
3. Plasma procainamide levels following a brief intravenous infusion were well described by a biexponential equation.
4. Therapeutic blood levels may be achieved with the sustained-release tablet with doses of approximately 1–2 g (four tablets) every 6–8 hr.

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# Liposomal Entrapment of Floxuridine


**STEPHEN P. SIMMONS and PAUL A. KRAMER [x]**



**Abstract** □ Floxuridine was found to have an apparent initial entrapment within negatively charged sphingomyelin liposomes about three times higher than its parent, fluorouracil. The drug also diffused out of the liposomes at a much lower rate than fluorouracil. Substitution of lecithin for sphingomyelin destroyed the effect. Liposomal entrapment may provide enhanced stability and decreased toxicity of floxuridine, permitting wider therapeutic utilization of pyrimidine nucleosides.



**Keyphrases** □ Floxuridine—entrapment within sphingomyelin and lecithin liposomes, compared to fluorouracil □ Liposomes, sphingomyelin and lecithin—entrapment of floxuridine, compared to fluorouracil □ Antitumor agents—floxuridine, entrapment within sphingomyelin and lecithin liposomes, compared to fluorouracil


The actual inhibitor of thymidylate synthetase and, hence, DNA biosynthesis in tumor growth inhibition by fluorouracil (I) is 2′-deoxy-5-fluorouridine 5′-monophosphate (II) (1). Floxuridine (2′-deoxy-5-fluorouridine) (III) is a better precursor of II than I, as evidenced by the fact that it is $10^3$ times as effective in inhibiting DNA thymine synthesis in Ehrlich ascites cells *in vitro* (2) and is a more effective carcinostatic agent against both Ehrlich ascites and Sarcoma-180 *in vivo* (3). This increased intrinsic activity is not translated into increased effectiveness in humans, because III is degraded to its constituent pyrimidine and sugar moieties by nucleoside phosphorylases in serum and tissues (4). Attempts at stabilization have included chemical modification of the drug itself as well as the addition of deoxyribose donors and enzyme inhibitors to suppress the degradation reaction (5, 6).

One might expect the encapsulation of III within the aqueous compartments of phospholipid vesicles (liposomes) to be of utility in this regard. The protective lipid sheath might prevent enzymatic degradation in serum and reduce the toxicity of the drug by excluding it from regions such as the GI tract. Previous workers reported difficulty

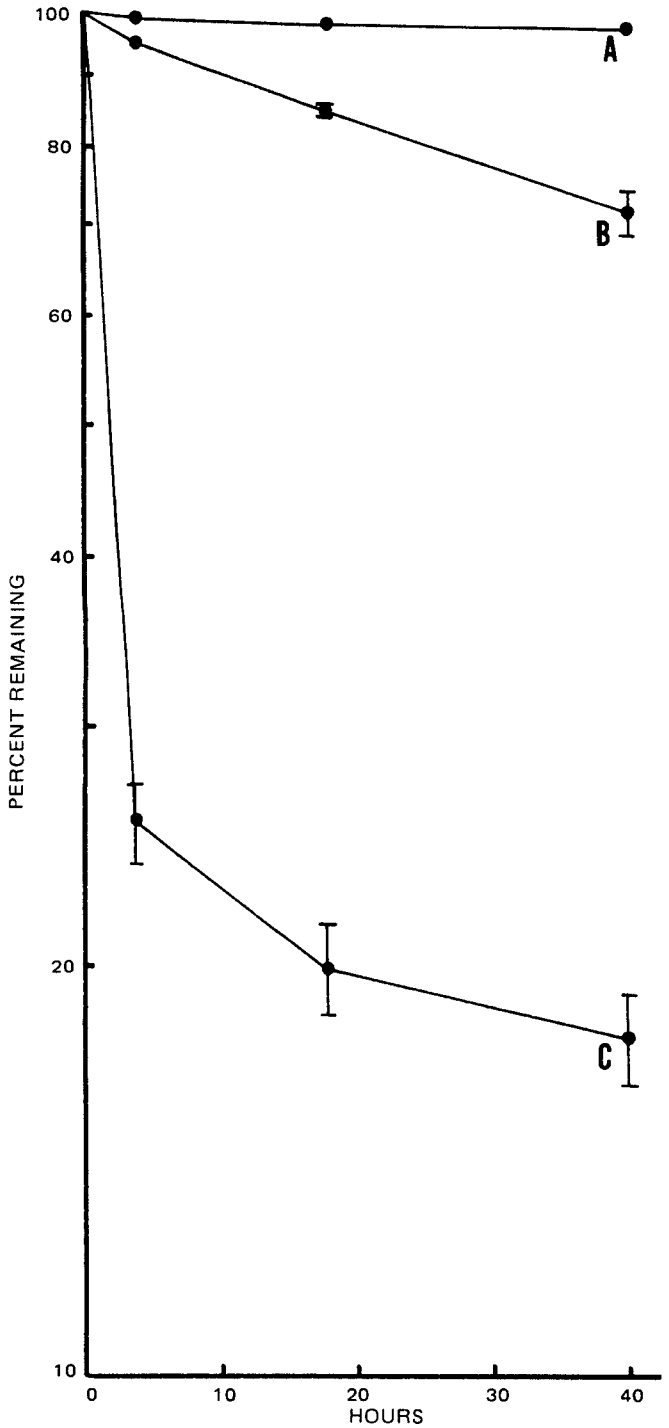


**Figure 1**—*Diffusion of sucrose (A), floxuridine (B), and fluorouracil (C) from sphingomyelin liposomes. The sphingomyelin–cholesterol–dicetyl phosphate ratio was 4.8:2.8:1.0, and the lipid–aqueous phase ratio was 70:1. The zero-time value of 100% represents initial entrapment after 4 hr of swelling at 25° plus 15 min of sonication. All other values are percentages of this initial quantity that remained entrapped after incubation of the liposomes for various times at 25°. Plotted points are mean ±SEM for three determinations.*

retaining I within liposomes (7, 8). The distribution of I in the rat was favorably altered toward exclusion of the drug from GI tissues, even though there was pronounced diffusion of drug from liposomes to plasma (8). The present studies were undertaken to determine if the more hydrophilic deoxyribose derivative might be a better candidate for liposomal delivery.

## EXPERIMENTAL

**Materials**—Sphingomyelin[1] (bovine brain), egg lecithin[2], and dicetyl phosphate[3] were used as received. Fluorouracil[4], floxuridine[5], sucrose[6], and cholesterol[5] were mixed with 6-$^{3}$H-fluorouracil[7] (26 Ci/mmole), 6-$^{3}$H-floxuridine[7] (2.3 Ci/mmole), 6,6′(*n*)-$^{3}$H-sucrose[7] (3.6 Ci/mmole), and 4-$^{14}$C-cholesterol[7] (47.2 mCi/mmole) (5 μCi/ml), respectively, prior to use. Spectral grade chloroform was used to dissolve the lipids, and all water was double distilled in an all-glass system.

**Liposome Preparation**—Liposomes were prepared from chloroform solutions of phospholipid (egg lecithin or sphingomyelin), cholesterol (sometimes 4-$^{14}$C-labeled), and dicetyl phosphate in molar ratios of 7:2:1 or 4.8:2.8:1. The lipids were evaporated under vacuum to a thin film on a 10-ml pear-shaped flask, and an isotonic, buffered (pH 6.0, 0.01 *M* phosphate) solution of tritiated sucrose, fluorouracil, or floxuridine (0.077 *M*) was added. The lipid–aqueous phase ratios used were 50 and 70 μmoles of lipid/ml of buffer.

A small number of 0.5-mm glass beads was added, and the lipids were dispersed by vortexing[8] for 2 min. After a 2-hr "swelling period" at 25°, the vesicles were sonicated in a bath sonicator[9] for 15 min at 15°. They were held at 25° for an additional 2 hr and then separated from unentrapped drug by chromatography on a 1.5 × 16-cm column[10]. The liposomal eluate was pooled, and the drug content was taken as a measure of "initial" entrapment. The liposomes obtained had a diameter range of 0.5–5.0 μm.

Quantitative determination of drugs and liposomal cholesterol was accomplished by liquid scintillation counting[11]. Drug release kinetics were determined by removing aliquots of liposomes from the pool at various sampling times, rechromatographing to remove released drug, and counting the liposome fractions upon elution from the column.

## RESULTS AND DISCUSSION

Table I shows the initial entrapment percentages as a function of drug and lipid composition of the liposome. These percentages depend upon lipid–aqueous phase ratios and molar ratios of constituents of the vesicle as expected. Sucrose was used as a nearly nondiffusable marker to indicate liposome integrity. Increasing the total lipid content and the relative quantity of cholesterol enhanced entrapment of both floxuridine and sucrose. These materials were entrapped to about the same extent when sphingomyelin was the phospholipid, but the entrapment of floxuridine relative to sucrose decreased dramatically when lecithin was substituted.

Increased unsaturation and melting of the acyl side chains were reported to increase the rate of diffusion of both charged and uncharged solutes through phospholipid membranes (9). The fact that sphingomyelin has a gel–liquid crystalline phase transition temperature of 40° (10) while egg lecithin vesicles "melt" at −15° (11) may account for the observed results. Fluorouracil was apparently entrapped within sphingomyelin liposomes about three times less extensively than floxuridine, but this finding could be an anomalous result produced by the arbitrary definition of "initial" entrapment.

Figure 1 depicts the release of sucrose, floxuridine, and fluorouracil from sphingomyelin liposomes at 25° over nearly 2 days. The liposomal preparation used was that providing the best initial entrapment (Table I). Sucrose was nearly completely retained, as expected. Fluorouracil diffused out with a very high initial rate, high enough to perhaps account for the apparently lower initial entrapment which was actually measured more than 4 hr after swelling began. At least for sphingomyelin liposomes, the hydrogen-bonding hydroxyl groups on the deoxyribose moiety of floxuridine prevent its rapid diffusional loss, as evidenced by the improved retention of floxuridine within sphingomyelin liposomes. This effect is in accord with previous observations by Cohen (12), who concluded that retention of materials within the aqueous interstices of liposomes was directly related to the ability of the material to hydrogen bond. The kinetics of diffusion of drug from these multiconcentric spheres

[1] Pierce Chemical Co., Rockford, Ill.
[2] Grand Island Biological Co., Grand Island, N.Y.
[3] K & K Labs, Plainview, N.Y.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Sigma Chemical Co., St. Louis, Mo.
[6] Mallinckrodt Chemical Works.
[7] Schwartz-Mann, Orangeburg, N.Y.
[8] Vortex-Genie, Scientific Industries, Springfield, Mass.
[9] Eighty watts, 60 Hz, Heat Systems Ultrasonics, Plainview, N.Y.
[10] Sephadex G-50 (coarse), Pharmacia Fine Chemicals, Piscataway, N.J.
[11] Beckman model LS-250.

**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.

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# 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