enriched diet markedly influenced the levels of cholesterol, the C/PL mole ratio and $\bar{\eta}$. The cholesterol level in the MML-treated animals, compared to old control rats, decreased in the hypothalamus ($P \leq 0.01$) and the hippocampus ($P \leq 0.005$) and attained in all brain regions at least the levels found in the young controls. The C/PL mole ratio of the MML-fed rats was lowered significantly in the hippocampus ($P \leq 0.01$) and cortex ($P \leq 0.005$) as compared to the old controls but did not approach, except for the hypothalamus ($P \leq 0.05$), the mole ratio of the young animals.

The changes in cholesterol and the C/PL mole ratios were accompanied by appropriate changes in $\bar{\eta}$. MML, when compared to the old animals, led to a decrease of $\bar{\eta}$ in the hypothalamus ($P \leq 0.005$), hippocampus ($P \leq 0.01$), and cortex ($P \leq 0.01$), and reached values approaching those of the young control rats.

The above data strongly indicate that MML notably influences the composition of brain membranes (cholesterol and phospholipids), and modulates $\bar{\eta}$. We do not yet know the permeability of MML through the blood–brain barrier, but on the basis of its results on the above brain properties it is reasonable to assume that MML reaches the brain via the blood stream. We also do not yet know the mechanism(s) of the MML effects which may act by extracting cholesterol [15] (as indicated by the lowered cholesterol level) or by incorporation of MML into responsive membranes [16]. Potent effects by an active lipid mixture on $\bar{\eta}$ and the C/PL mole ratio have been reported in a number of papers [10]. The present work shows that pronounced modulating effects on cholesterol level, C/PL mole ratio, and $\bar{\eta}$ in at least the hippocampal and cortex regions of rat brain cell can be achieved by a diet with a lipid fraction prepared from plant sources. Detailed analytical studies of the various components of PL in the tissue samples and physiological and psychological implications of these findings are in progress.

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Biological activity of two novel inhibitors of uridine phosphorylase

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Inhibitors of pyrimidine nucleoside phosphorylases have been recognized as potential chemotherapeutic agents by virtue of their inhibition of pyrimidine salvage and by their modification of the metabolism of other chemotherapeutic agents [1–3]. The two mammalian pyrimidine nucleoside phosphorylases, uridine phosphorylase (UrdPase, EC 2.4.2.3) and thymidine phosphorylase (dThdPase, EC 2.3.2.4) share many common natural and synthetic substrates [1, 4], with a notable exception that uridine and 5-fluorouridine are substrates for UrdPase but not dThdPase [1]. Niedzwicki *et al.* [1, 5] reported the synthesis of several 5-substituted acyclic uridine derivatives which were more potent inhibitors of UrdPase than the corresponding uridine derivatives. Efficacy of these inhibitors is related to the hydrophobicity of the 5-substituent, for which a binding site exists on UrdPase [5, 6]. Thus, the solubility of these compounds is often quite poor in aqueous systems ([2] and Table 1). We report two new highly water-soluble acyclic nucleoside analogs with potent UrdPase inhibitory activity.

Materials and methods

5-Benzylacylcouridine (BAU),1-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-5-benzyluracil (DHPBU), and (RS)-1-[[2-hydroxy-1-(aminomethyl)ethoxy]methyl]-5-benzyluracil (AHPBU) were synthesized in our laboratory.* [2-^{14}C]Uridine (56 mCi/mmole) and [2-^{14}C]thymidine (56 mCi/mmole) were purchased from Moravek Biochemicals, Brea, CA. UrdPase from S-180 cytosolic extract was prepared as previously described [1]. Human

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platelets were isolated from a healthy male donor, and dThdPase activity from both intact and lysed (0.5% Triton X-100) platelets was assayed. The pyrimidine nucleoside phosphorylase in S-180 cells is only UrdPase [5] and in platelets only dThdPase [7]. Solubilities in water at 25° were determined by u.v. spectroscopy [8]. UrdPase and dThdPase activities were assayed at pH 8.0 in 20 mM potassium phosphase, 1 mM EDTA, 1 mM β-mercaptoethanol containing 26 μg S-180 cytosolic protein or approximately 3×10^8 intact or lysed platelets. Reactions were preincubated at 37° for 5 min and initiated by the addition of 25–300 μM substrate containing 0.3 μCi [2-^{14}C]-labeled uridine or thymidine per 200 μl reaction mixture. The desired concentration of inhibitor was added to the appropriate reaction mixtures prior to preincubation. A 30-μl aliquot was withdrawn from each reaction mixture at 0, 20, 40 and 60 min after initiating the reaction, and each was added to tubes containing 10 μl of 40% perchloric acid. After neutralization with 10 μl of 10 N KOH, the aliquots were chromatographed by high performance liquid chromatography (HPLC) on a 10 μm Lichrosorb RP-18 column (25 × 4.6 cm, Rainin Instrument Co., Inc., Woburn, MA). The isocratic elution utilized 0.1 M sodium phosphate, 2.5% methanol, pH 6.4, at a flow rate of 0.7 ml/min and 25° to separate uridine and uracil. Thymidine and thymine were separated by the adjustment of the mobile phase to 5% methanol. Pyrimidine base and the corresponding nucleoside eluted at 12 and 27 min respectively. Quantitation of the [2-^{14}C]uracil or [2-^{14}C]thymine formed was accomplished by continuous monitoring of the radioactive eluent by a Flo-One radioactive flow detector (Radiomatic, Inc., Tampa, FL) equipped with a 2.5 ml liquid scintillant flow cell. Monofluor (National Diagnostics, Sommerville, NJ) or Aquasol (New England Nuclear, Boston, MA) was used as scintillant and pumped at a rate of 4.0 ml/min through the flow cell.

The velocity of the UrdPase reaction was proportional to enzyme concentration up to at least 125 μg S-180 cytosolic protein per assay, and reaction velocities were linear to at least 90 min. The average K_m for uridine at pH 8.0 was 89 ± 18 μM and was derived from five independent determinations. Individual K_m and $K_{m,app}$ values were calculated by the statistical method of Wilkinson [9], and the K_i values reported represent the mean ± S.D. of determinations at three inhibitor concentrations. Niedzwicki *et al.* [1] report a K_m for uridine of 0.1 mM utilizing S-180 cytosol at pH 8.0.

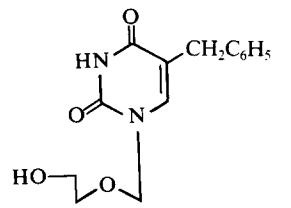
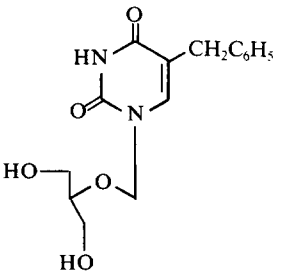
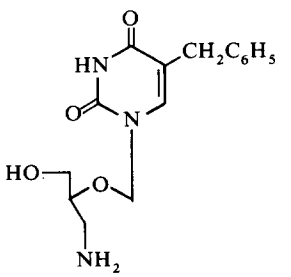
Results and discussion

The structures and relative solubilities of BAU, DHPBU, and AHPBU are shown in Table 1. The addition of a second hydroxymethyl group to the acyclic moiety enhanced the water solubility over that of BAU *ca.* 150-fold, whereas the addition of an aminomethyl group enhanced solubility even more. BAU is inactive against dThdPase [1], and similarly DHPBU or AHPBU, up to 100 μM, did not inhibit the hydrolysis of thymidine by either intact or lysed human platelet preparations. BAU is, however, a potent competitive inhibitor of UrdPase with a K_i of 0.098 μM [1]. DHPBU and AHPBU were also competitive inhibitors of UrdPase, with K_i values of 0.098 ± 0.032 and 0.020 ± 0.009 μM respectively (Figs. 1 and 2). DHPBU is apparently non-cytotoxic to L1210 and S-180 cells as no alteration in growth rate was observed up to a 1 mM concentration. AHPBU induced some (*ca.* 20%) reduction in the growth rate of S-180 cells at a concentration of 1 mM, which is 50,000-fold in excess of K_i and hence would probably not be a pharmacological problem *in vivo*.

This study indicates that it is possible to drastically increase the water solubility of uridine analogs substituted in the 5-position of the uracil moiety without compromising binding affinities to UrdPase. Indeed, addition of an aminomethyl group to BAU enhances its binding affinity to S-180 UrdPase in addition to increasing solubility. Studies are in progress to determine if these novel inhibitors potentiate the toxicity of other chemotherapeutic agents. Synthesis of novel pyrimidine phosphorylase inhibitors containing these acyclic moieties conjugated to other base analogs may lead to new chemotherapeutic agents with broader inhibitory activity yet high solubility. DHPBU and AHPBU thus represent a new class of potent UrdPase inhibitors: acyclic uridine derivatives with a hydrophobic substitution at the C-5 position but with relatively high water solubility.

Addendum—Subsequent to the completion of this manuscript, we learned of an independent report of the synthesis and activity of DHPBU [10]. The *in vitro* antineoplastic activity of 5-fluorodeoxyuridine was potentiated significantly by DHPBU [10], and this confirms that further study of the chemotherapeutic efficacy of DHPBU, AHPBU, and related compounds is warranted.

Table 1. Structures and relative solubilities of BAU, DHPBU, and AHPBU

Compound		Solubility*
BAU		1.8
DHPBU		270
AHPBU		>300†

* Solubility: mg/ml H_2O at 25° by an ultraviolet spectroscopic method.

† Solubility determination was limited by availability of compound.

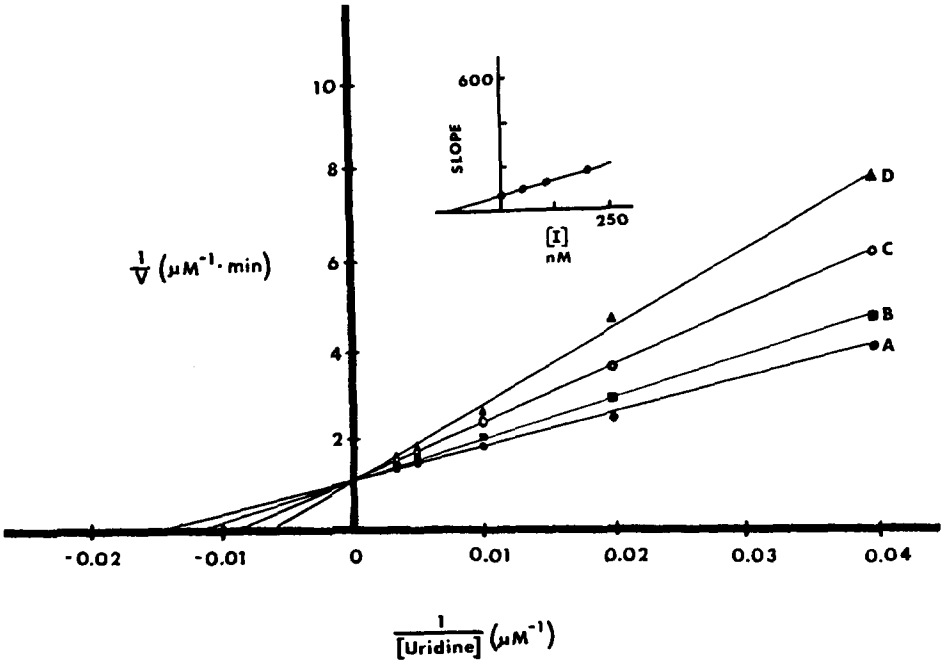


Fig. 1. Double-reciprocal plot for inhibition of uridine phosphorylase by DHPBU. Key: (A) no DHPBU; (B) 0.05 μM DHPBU; (C) 0.10 μM DHPBU; and (D) 0.20 μM DHPBU. Each velocity determination contained 26 μg S-180 cytosol protein per assay.

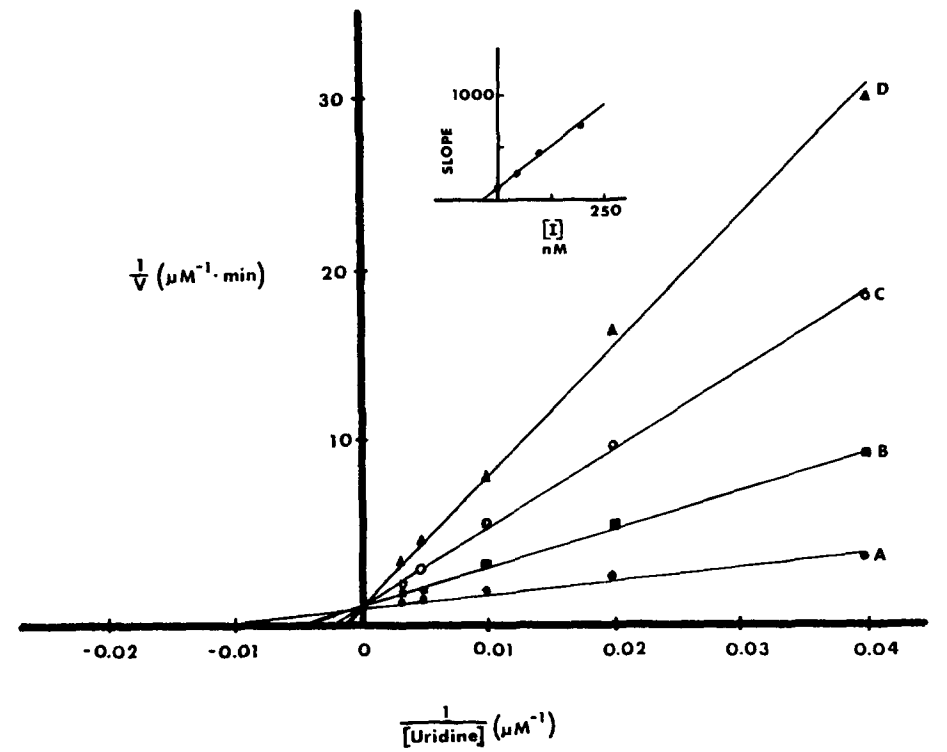


Fig. 2. Double-reciprocal plot for inhibition of uridine phosphorylase by AHPBU. Key: (A) no AHPBU; (B) 0.05 μM AHPBU; (C) 0.10 μM AHPBU; and (D) 0.20 μM AHPBU. Each velocity determination contained 26 μg S-180 cytosol protein per assay.

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