in nucleoside permeation, and (d) variation in the sensitivity of the nucleoside-transport mechanisms to NBMPR among different cell types. The existence in particular lines of cultured neoplastic cells of nucleoside-transport mechanisms of low sensitivity to NBMPR has been recognized recently [11, 12]. One instance is known of a line of neoplastic cells, the Walker 256 carcinosarcoma, which lacks NBMPR-binding sites and possesses a nucleoside-transport mechanism that is insensitive to NBMPR [13].

In conclusion, this study indicates that prior i.p. treatment of mice with NBMPR-P, a prodrug form of the potent inhibitor of nucleoside transport, NBMPR, had only a modest inhibitory effect on the salvage of circulating uridine in several tissues and increased uridine salvage by 63% in kidney.

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Uracil enhancement of 5-fluorodeoxyuridine incorporation into human breast carcinoma deoxyribonucleic acid*

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The cytotoxicity of 5-fluorouracil (FUra) and 5-fluorodeoxyuridine (FdUrd) has been ascribed to the inhibition of thymidylate synthetase by 5-fluorodeoxyuridine monophosphate [1, 2]. The fluorinated pyrimidines also incorporate into RNA and disrupt RNA processing [3–7]. The relative importance of each cytotoxic mechanism may be dependent upon varying patterns of intracellular FUra metabolism [8]. The incorporation of FUra into MCF-7 human breast carcinoma cellular RNA, however, correlates with loss of clonogenic survival [9]. This relationship is also maintained when inhibition of DNA synthesis is reversed by thymidine [9]. These observations suggest that the formation of (FUra)RNA is a major mechanism of cytotoxic action.

FUra residues have also been detected in eukaryotic DNA [10–13]. The FUra residues are excised from DNA [14], and this excision may contribute to the cytotoxicity associated with formation of (FUra)DNA [15]. There are at least two mechanisms that limit the incorporation of FUra into eukaryotic DNA. Deoxyuridine-triphosphate nucleotidohydrolase degrades intracellular FdUTP, and uracil-DNA glycosylase removes FUra residues incorporated in the DNA strand [16]. The excision of FUra from DNA by uracil-DNA glycosylase is much less efficient than that of uracil [17], and this enzyme is inhibited by uracil [18–20].

In view of the inhibition of uracil-DNA glycosylase by uracil and the previous demonstration that FUra residues are excised from MCF-7 DNA, we have studied the effects of uracil on the formation of (FUra)DNA. The results demonstrate that uracil enhances the incorporation of FUra into MCF-7 DNA.

Materials and methods

Cell culture. The human breast carcinoma MCF-7 cell line was obtained from the Michigan Cancer Foundation, Detroit. MI. The cells were grown free of Mycoplasma contamination as a monolayer in Dulbecco's Modified Eagle's medium (Grand Island Biological Co., Grand Island, NY) with 10% heat-inactivated dialyzed fetal calf serum, 1% L-glutamine, 100 μg penicillin/ml, 10 μg insulin/ml, and 100 units streptomycin/ml.

Incorporation of FUra into nucleic acids. MCF-7 cells in logarithmic growth phase at a concentration of 1×10^6 cells/100×20 mm tissue culture dish (Costar Plastics, Cambridge, MA) were washed twice with serum-free medium and incubated with 10^{-7}, 10^{-6}, or 10^{-5} M [^{3}H]FdUrd (18 Ci/mmole; Moravek Biochemicals, City of Industry, CA) and 10 μCi $H_3{}^{32}PO_4$ (carrier-free; New England Nuclear Corp., Boston, MA) for 3 or 6 hr. Uracil (Sigma Chemical Co., St. Louis, MO) was added at concentrations of 0.015, 0.15, 1.5 and 15 mM. The total cellular nucleic acids were purified as previously described [9] and analyzed by cesium sulfate gradient centrifugation.

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Results

The effects of uracil on the incorporation of [^{3}H]FdUrd into MCF-7 DNA and RNA are shown in Fig. 1. MCF-7 cells were incubated for 3 hr with 10^{-5} M [^{3}H]FdUrd alone (Fig. 1A) and with 1.5 mM uracil (Fig. 1B). The nucleic acids were extracted and analyzed by Cs_2SO_4 density gradient centrifugation which separates RNA (banding at 1.65 g/ml) and DNA (banding at 1.45 g/ml). The labeling with ^{32}P provides a measure of the relative rates of RNA and DNA synthesis. The incorporation of ^{32}P (Fig. 1) indicates that the synthesis of these nucleic acids was not inhibited significantly by 1.5 mM uracil. The incorporation of [^{3}H]FUra residues into RNA was similar in the presence or absence of uracil, while uracil treatment resulted in an enhancement of the tritium radioactivity incorporated into DNA. As we have demonstrated previously, the tritium detectable in MCF-7 DNA represents the specific incorporation of FUra residues [13]. The extent of [^{3}H]FUra residues detectable in DNA after a 3-hr incubation in 10^{-5} M [^{3}H]FdUrd was enhanced by 1.83-fold as a result of 1.5 mM uracil exposure (Table 1). Similar experiments were performed using 10^{-7} M and 10^{-6} M [^{3}H]FdUrd, and the formation of (FUra)DNA was similarly enhanced by uracil (Table 1).

The effects of various concentrations of uracil on the incorporation of 10^{-6} M [^{3}H]FdUrd into DNA were then monitored using cesium sulfate gradient centrifugation. The results obtained are illustrated in Fig. 2. MCF-7 cells exposed to increasing concentrations of uracil for 6 hr formed progressively more (FUra)DNA. In this series of experiments, 1.5 and 15 mM uracil enhanced FUra incorporation into DNA by 3- to 4-fold.

Table 1. FdUrd incorporation into MCF-7 DNA

FdUrd [M]	Control*	1.5 mM Uracil	Enhancement
10^{-7}	0.013	0.024	1.85×
10^{-6}	0.145	0.248	1.71×
10^{-5}	1.750	3.210	1.83×

*Results are expressed as pmoles FdUrd incorporated into DNA/10^6 cells.

Discussion

Although FUra residues have been detected in bacterial DNA [21], this finding has only recently been established for eukaryotic DNA [10–13]. Previous attempts had employed low specific activity [^{14}C]FUra [22], and different cell lines may vary in the ability to incorporate FUra into DNA based upon the intracellular levels of uracil-DNA glycosylase and nucleotide hydrolase.

We have demonstrated previously that FUra incorporates into MCF-7 human breast carcinoma DNA [13]. We have also demonstrated the FUra residues are excised from MCF-7 DNA and that methotrexate enhances the excision process [14]. The enhancement by methotrexate may be related to the induction of increased levels of uracil-DNA glycosylase. The present studies extend these findings by demonstrating that uracil, an inhibitor of uracil DNA

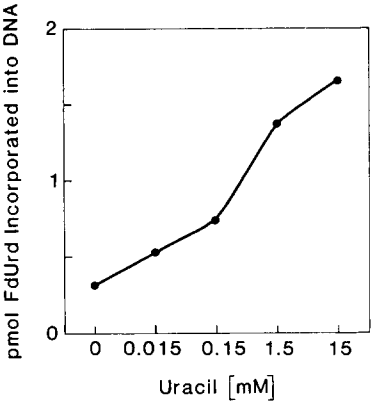

Fig. 2. Effect of various concentrations of uracil on [^{3}H] FdUrd incorporation into MCF-7 DNA. MCF-7 cells were incubated with 10^{-6} M [^{3}H]FdUrd and various concentrations of uracil for 6 hr. Total cellular nucleic acids were purified and analyzed by cesium sulfate gradient centrifugation. The results are expressed as the pmoles of FdUrd incorporated into DNA/10^6 cells.

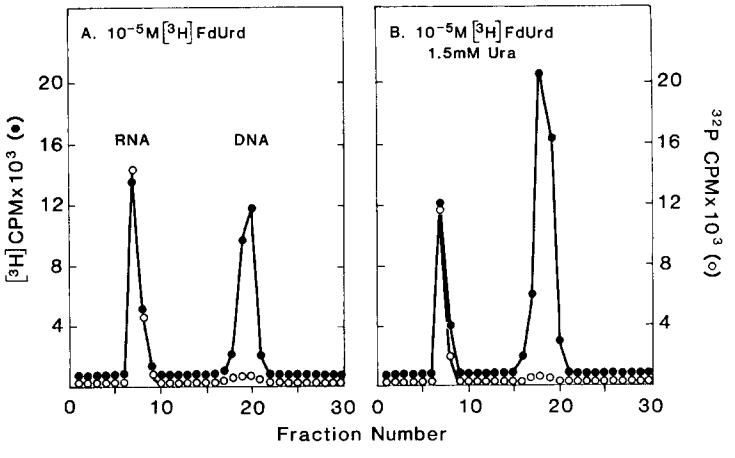

Fig. 1. Effect of 1.5 mM uracil on incorporation of [^{3}H]FdUrd into MCF-7 nucleic acids. MCF-7 cells in logarithmic growth phase were incubated with 10^{-5} M [^{3}H]FdUrd and 10 μCi/ml ^{32}P for 3 hr. Total cellular nucleic acids were purified and analyzed by cesium sulfate density gradient centrifugation. Key (A) control; and (B) 1.5 mM uracil.

glycosylase [18–20], enhanced the formation of (FUra)-DNA. A similar 3- to 5-fold increase in (Ura)DNA formation occurs when human lymphoblast uracil-DNA glycosylase is inhibited by uracil [23]. Although uracil treatment may result in higher intracellular levels of FUra containing nucleotides and thus greater incorporation into DNA, the addition of similar concentrations of uracil in a previous study had no effect on intracellular nucleotide pools [23]. Further, uracil did not enhance incorporation of FUra into RNA.

Uracil-DNA glycosylase initiates a process of excision and repair by producing an apyrimidinic site following removal of FUra [24]. This excision could result in DNA fragmentation. A recent study has demonstrated that treatment with FdUrd results in small size DNA fragments as analyzed by alkaline sucrose gradient centrifugation [25]. This finding could be secondary to excision of Fura residues or to the inhibition of DNA synthesis by this agent. It should now be possible to employ uracil to inhibit the excision of FUra residues and determine whether there is an associated decrease in DNA fragmentation. It would also be of interest to determine whether uracil prevents the cytotoxicity associated with incorporation of FUra residues in DNA [15].

In summary, recent works [13] has demonstrated that FdUrd misincorporates into MCF-7 human breast carcinoma DNA. The incorporated FUra residues are partially excised from MCF-7 DNA [14] and this may contribute to the cytotoxicity associated with (FUra)DNA formation [15]. We have attempted to extend these findings by monitoring the effect of uracil on the extent of FUra misincorporation into DNA. The results demonstrate that uracil enhances the formation of MCF-7 (FUra)DNA.

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Morphine dependence and withdrawal without alterations in cerebral β-adrenergic receptor density*

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Opiate analgesics appear to have direct effects on catecholamine-containing neurons. Morphine decreases norepinephrine release from sympathetic neurons in the cat nictitating membrane [1] and in the mouse vas deferens [2]. In the brain, opiates decrease the firing of noradrenergic neurons in the locus coeruleus [3], increase catecholamine synthesis [4, 5], and decrease the depolarization-induced release of norepinephrine from cerebellar cortex [6]. Since clonidine, a selective α_2-adrenergic receptor agonist, can partially suppress morphine withdrawal [7], it has been suggested that alterations in the activity of noradrenergic neurons might play a role in physical dependence on morphine. Similarities in the electrophysiological actions of clonidine and morphine have been noted [8, 9], and chronic morphine administration has been shown to increase the apparent number of [^{3}H]

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