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^{19}F Nuclear Magnetic Resonance Analysis of 5-Fluorouracil Metabolism in Wild-Type and 5-Fluorouracil-Resistant *Nectria haematococca*

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A mutant (*furA3*) was isolated from the *S1* wild-type strain of *Nectria haematococca* on the basis of its resistance to 5-fluorouracil (5FU). This mutant has greatly reduced activity of uracil phosphoribosyltransferase, a pyrimidine salvage enzyme catalyzing the synthesis of UMP from uracil. The metabolism of 5FU was examined in both strains by using ^{19}F nuclear magnetic resonance spectroscopy. In the *S1* strain, 5FU appears to be metabolized by two pathways operating simultaneously: (i) conversion to fluoronucleotides and (ii) degradation into α-fluoro-β-alanine. The *furA3* mutant shows metabolic changes consistent with a uracil phosphoribosyltransferase lesion, since it takes up 5FU and forms a small amount of α-fluoro-β-alanine but does not synthesize fluoronucleotides. Since pigment synthesis is strongly enhanced by 5FU in the *S1* wild-type strain but not in the *furA3* mutant, these results support the hypothesis that 5FU stimulation of secondary metabolism in *N. haematococca* is not mediated by the drug itself but involves a phosphorylated anabolite.

Previous work (19) showed that the pyrimidine analog 5-fluorouracil (5FU) strongly stimulated naphthoquinone production by *Nectria haematococca* (Berk. and Br.) Wr, the sexual form of the filamentous fungus *Fusarium solani*, when added to culture media before the end of the exponential growth phase. 5FU and other fluorinated pyrimidines are known to interfere with nucleotide metabolism of a wide variety of living species, leading to reduced rates of many biosynthetic processes and reduced growth (9, 16, 22, 24, 28). Because of these properties, fluoropyrimidines have been widely used clinically in cancer chemotherapy, for treatment of fungal infections (9, 21, 25), and also in experimental research to assess the role of nucleic acids and proteins in a number of biological processes (6, 10, 14, 18). However, very few studies describing stimulation of a biosynthetic pathway as a response to fluoropyrimidine exposure have thus far been reported (19, 23).

The analysis of 5FU metabolism in *N. haematococca* is presently a prerequisite for the elucidation of the mechanism(s) by which this antimetabolite enhances naphthoquinone production. ^{19}F nuclear magnetic resonance (NMR) spectroscopy is a particularly powerful tool for the investigation of fluoropyrimidine metabolism, since it allows simultaneous detection of all fluorinated compounds present at a given time. It has already been exploited to characterize, noninvasively, 5FU and related products in living bacteria (8), yeasts (4, 26), and mammalian cells (13, 15). In order to determine whether ^{19}F NMR spectroscopy can be used for monitoring 5FU metabolism in *N. haematococca*, the patterns of fluorinated compounds were examined at various times in cultures of both a 5FU-resistant mutant and the wild-type strain.

MATERIALS AND METHODS

Chemicals. 5-Fluorouridine (FUrd) and 5-fluoro-2′-deoxyuridine (FdUrd) were generously supplied by Hoffmann-La Roche Inc., Basel, Switzerland. 5-Fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) and 5-fluorouridine-5′-triphosphate (FUTP) were purchased from Sierra Bioresearch, Tucson, Ariz. 5FU was from Sigma Chemical Co., St. Louis, Mo., and α-fluoro-β-alanine (FBAL) was from Koch-Light Laboratories, Colnbrook, United Kingdom. Chromium (III) acetylacetonate [$Cr(acac)_3$] was a product of Spectrometrie Spin Techniques, Paris, France. 4-Fluorobenzoate was prepared by titrating 4-fluorobenzoic acid (Fluka, Buchs, Switzerland) with an NaOH solution.

Strains and cultivation methods. The *furA3* mutant was derived from the *S1* homothallic wild-type strain of *N. haematococca* (20). Stock cultures were maintained on potato dextrose agar at 26°C. Spontaneous mutants resistant to 5FU were obtained by plating 10^5 *S1* microconidia per petri dish on synthetic medium agar (MSA) (19) containing 3.8 mM 5FU. Testing of 5FU resistance was done on plates of MSA to which increasing amounts of 5FU were added. The selected mutants were crossed with tester strains to determine the genetical basis of resistance, and the progenies were analyzed by using random ascospore analysis (20).

Determination of UPRTase activity. Mycelium for enzyme extraction was grown for 48 h at 26°C on MSA plates layered with cellophane (19). The mycelia from two petri dishes (0.9 to 1.1 g [fresh weight]) were scraped off, frozen in liquid nitrogen, and ground in a cold mortar. The powder was transferred to a centrifuge tube and allowed to thaw in 4 ml of extraction buffer (50 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], 1 mM EDTA, 6.5 mM dithiothreitol, pH 7.0) and was homogenized in the extraction buffer. The homogenates were centrifuged at 25,000 × *g* for 15 min at 4°C, and the supernatants were immediately used for enzyme analysis. The uracil phosphoribosyltransferase (UPRTase) assays were carried out in microcentrifuge tubes. A 50-μl portion of supernatant (containing about 30 μg of protein) was added to 100 μl of a reaction mixture prepared according to the method of Jones (11). Blanks were set up by using supernatant aliquots previously boiled for 10 min. The reaction tubes were maintained at 30°C for 1 h in a water bath. The reaction was stopped by heating the tubes

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for 10 min at 95°C. After cooling in ice, 10-μl portions of reaction mixtures were withdrawn and spotted on polyethyleneimine-cellulose sheets (no. 5579; Merck & Co., Inc., Rahway, N.J.), along with 10 μl of nonradioactive uridine, uracil, UMP, UDP, and UTP standards (0.5 mg/ml). The plates were developed for 70 min with H_2O and then for 20 min with 0.55 M LiCl at about 20°C. Spots were visualized under short-wave UV light (260 nm), and radioactivity on them was determined by the method of Jones (11). Protein concentrations were determined by the method of Bradford (1), with bovine serum albumin as the standard.

Preparation of samples for ^{19}F NMR analysis. Mycelium was grown on MSA medium containing either 76.9 or 769 μM 5FU and was harvested after appropriate incubation times. Spectra of living fungal cells were obtained by gently packing about 2 g of mycelium, previously washed with sodium phosphate buffer (10 mM, pH 6), into the NMR tube. After in vivo NMR recordings, the samples were collected, cooled to +4°C, and extracted with 10% perchloric acid (PCA) by using the method of Wain and Staatz (27). The cold and the hot PCA extracts sequentially obtained from each sample were both concentrated in vacuo to a final volume of 3 ml, and their pH was adjusted to 5 to 5.5. About 2 mg of the relaxation reagent, $Cr(acac)_3$, was then added. Portions of agar culture media were melted, transferred into NMR tubes, and analyzed after cooling to room temperature. In some experiments the agar medium was frozen at −20°C and then thawed, and the exudate thus obtained was analyzed. The identification of the fluoronucleosides present in the culture media was performed according to the methods described by Dreyer and Cadman (5) and Garrett and Santi (7): 100 μl of 0.5 M $NaIO_4$ prepared daily and 125 μl of a 4 M solution of methylamine which had been slowly brought to pH 7.5 with H_3PO_4 and was added several minutes after the $NaIO_4$ were added to 3 ml of the neutralized exudate (pH 6.5 to 7.5). After being mixed, the sample was incubated at 37°C for 40 min. The remaining IO_4^- was destroyed by adding 25 μl of 1 M rhamnose. The sample was acidified to pH 5.0 to 5.5, and the ^{19}F NMR spectrum was recorded.

^{19}F NMR analysis. Proton-decoupled ^{19}F NMR spectra were recorded at 282.4 MHz on a Bruker WB-AM 300 spectrometer by using 10-mm diameter NMR tubes. The magnetic field was shimmed by using the ^{1}H NMR resonance of water. The chemical shifts (δ) were reported relative to the resonance peak of CF_3COOH (5% aqueous solution, wt/vol) as an external reference. Spectra were run in the following instrumental conditions: probe temperature, 25°C; sweep width, 29,411 Hz; 32,768 data points zero filled to 65,536; pulse width, 7 μs (i.e., flip angle, ≈40°); repetition time, 1 s for living mycelia and culture media and 3 s for PCA extracts; number of scans, 10,000 to 15,000 for living mycelia and PCA extracts and 2,000 for culture media; line broadening caused by exponential multiplication, 10 Hz for living mycelia and agar media, 3 Hz for PCA extracts, 1 Hz for frozen-thawed media. For the quantification of PCA extracts, a capillary containing a solution of 4-fluorobenzoate and $Cr(acac)_3$ in D_2O was inserted coaxially in the NMR tube. This reference solution was calibrated against known standards of 5FU and FBAL doped with $Cr(acac)_3$ in the recording conditions used for PCA extracts. The concentrations of the fluorinated metabolites were measured by comparing the expanded areas of their respective NMR signals with that of 4-fluorobenzoate. The areas were determined after the different signals were cut out and weighed. Peak assignments were done by adding standard fluorinated compounds to culture media or PCA fungal extracts.

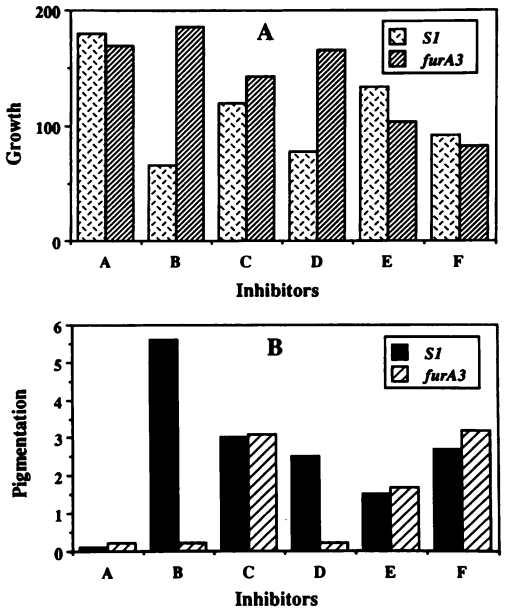

FIG. 1. Influence of five antimetabolites on growth and pigmentation of the *S1* and *furA3* strains of *N. haematococca*. A, No antimetabolite (control); B, 5FU (38 μM); C, FUrd (170 μM); D, 6-azauracil (400 μM); E, 5-fluoro-DL-tryptophan (450 μM); F, cycloheximide (355 μM). Growth expressed as milligram of dry mycelium per petri dish (A): ⊠, *S1*; and ▨, *furA3*. Pigmentation expressed as the absorbance at 490 nm of a 50-ml aqueous extract of the medium from one petri dish (B) (19): ■, *S1*; and ▨, *furA3*. Age of cultures, 10 days.

RESULTS AND DISCUSSION

Isolation and characterization of the *furA3* mutant. On MSA medium, the *S1* wild-type strain grows well and is only lightly pigmented. In the presence of 5FU concentrations ranging from 38 to 380 μM, *S1* growth is reduced by about 66% and the cultures accumulate naphthoquinones (19). Higher concentrations (380 μM to 3.8 mM) lowered growth without arresting it: 769 μM, 1.5 mM, and 3.8 mM 5FU reduced the growth of the *S1* strain to about 28, 23, and 19% of that of the untreated control, respectively. The *furA3* mutant was isolated as a fast-growing colony from the weak background growth obtained by plating *S1* microconidia on MSA containing 3.8 mM 5FU. It was purified by three rounds of single-microconidium isolation on the same medium. The *furA3* mutant grows well and remains lightly pigmented in the presence of 5FU levels up to 10 mM. It was also fully resistant to 6-azauracil but as sensitive as the parental *S1* strain to FUrd, cycloheximide, and 5-fluoro-DL-tryptophan (Fig. 1). It proved to be stable after serial microconidial transfers on media without 5FU, and no back mutation occurred. The *furA3* mutant was crossed with 5FU-sensitive strains derived from *S1* and carrying various markers suitable for genetic analysis (20). The progenies consisted of approximately equal numbers of 5FU resistant- and 5FU-sensitive isolates, showing that 5FU resistance segregated as a single gene difference. The *furA3* mutation was found linked to *met9*, a mutation resulting in methionine requirement. The *furA* and *metA* genes were 16 map units apart.

Previous work showed that the *furA3* mutant did not incorporate radioactive label from [2-^{14}C]5FU into trichloroacetic acid-insoluble material (19). This finding and the

TABLE 1. Uracil phosphoribosyltransferase activity of 2-day-old *S1* and *furA3* strains of *N. haematococca*

Strain	Percentage of initial radioactivity[a] found in:		
	Uracil	UMP	Uridine
S1	26	55	18
furA3	90	9	1

[a] Initial radioactivity brought as [2-^{14}C]uracil (5.5 mCi/mmol).

growth responses of *furA3* to pyrimidine analogs indicate some similarity between this strain of *N. haematococca* and the 5FU-resistant, FUrd-sensitive mutants of *Neurospora crassa* (2, 3), *Saccharomyces cerevisiae* (12), and *Aspergillus nidulans* (17) lacking UPRTase activity. UPRTase levels in wild-type and *furA3* mycelia grown on MSA were determined. From the results summarized in Table 1, it will be seen that the activity detected in *furA3* mycelium is less than 20% of the activity of the wild type.

^{19}F NMR spectroscopy of *N. haematococca* wild-type and *furA3* mutant strains. ^{19}F NMR spectroscopy was used to compare the abilities of wild-type and *furA3* mycelia to convert 5FU into intracellular fluorinated metabolites and to identify these metabolites. Since NMR is inherently a relatively insensitive technique, preliminary experiments were conducted with a high dose of 5FU in order to see if fluorinated compounds could be detected.

(i) ^{19}F NMR study of both strains grown on MSA containing 769 μM 5FU. Figure 2 shows the ^{19}F NMR spectra of living mycelia of both strains after 3 days for *S1* or 4 days for *furA3* on MSA containing 769 μmoles of 5FU per liter. Two broad peaks in a 1/1 ratio (δ = −88.8 and −93.3 ppm) were observed in the *S1* spectrum (Fig. 2A), whereas only the signal at −93.3 ppm was present in the *furA3* spectrum (Fig. 2B). From the values of their chemical shifts, the peaks at −88.8 and −93.3 ppm were assigned, respectively, to intracellular free fluoronucleotides (FNUCt) and unmetabolized 5FU (26). However, the signal at −88.8 ppm being very broad, it could eventually mask a small signal corresponding to fluoronucleosides (FNUCs) that resonate ≈1 ppm more shielded (26). It was therefore called FNUC, i.e., FNUCt plus possibly FNUCs. The proportion of FNUC relative to 5FU was increased (3/1 ratio) when a living mycelium of the *S1* strain was studied after 5 days on MSA containing 769 μmoles of 5FU per liter (data not shown).

Samples of the nonseeded medium and of media which supported growth of each strain were melted, and their ^{19}F NMR spectra were recorded at 25°C. The signal of 5FU (−93.3 ppm) was observed in each spectrum. Moreover, a small peak attributed to FNUCs (−89.9 ppm) was detected in the 5-day-old culture medium of the *S1* strain (data not shown). This signal did not correspond to a degradation product that could be formed when the medium was heated, since it was also present in the frozen-thawed sample of the same batch of medium.

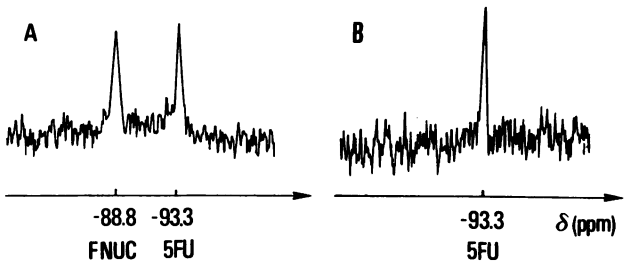

FIG. 2. ^{19}F NMR spectra of living mycelia of *S1* strain after 3 days (A) and *furA3* strain after 4 days (B) on MSA containing 769 μmoles of 5FU per liter.

(ii) ^{19}F NMR study of the *S1* strain grown on MSA containing 76.9 μM 5FU. After these encouraging results, a more detailed study was conducted with a less toxic dose of 5FU closer to that used for naphthoquinone production, i.e., 76.9 μmoles of 5FU per liter. Spectra from living mycelia of the *S1* strain grown for 3 and 4.5 days on MSA showed the resonances of FNUC (−88.8 ppm), 5FU (−93.3 ppm), and FBAL (−112.6 ppm) (Fig. 3A$_1$). Only the signal of FNUC could be detected in the living mycelium of the *S1* strain grown for 8 days (Fig. 3A$_2$).

Spectra from the corresponding media consisted of a major peak of 5FU (−93.3 ppm) and a signal for FNUCs (−89.9 ppm) (Fig. 3B). The peak of FBAL (−112.6 ppm) appeared only in the 8-day-old culture medium (Fig. 3B$_2$). This suggests that intracellular FBAL synthesized during the growth phase was released into the substrate by the old hyphae and explains the disappearance of FBAL from the 8-day-old *S1* mycelium (Fig. 3A$_2$). Since the two FNUCs, FUrd and FdUrd, have nearly identical chemical shifts (26), three experiments were necessary for an unambiguous assignment of the FNUCs signal at −89.9 ppm to FUrd. The ^{19}F NMR spectrum of a sample of frozen-thawed culture medium after addition of FdUrd showed two signals separated by 3.7 Hz. When FUrd was then added, the downfield signal was increased. So, the FNUCs found in culture media is FUrd. To confirm this, a third experiment was done. It is known that a treatment with periodate and methylamine leads to a complete destruction of ribonucleosides and ribonucleotides into corresponding base but is ineffective on the corresponding deoxy derivatives (5, 7). When a frozen-thawed culture medium was treated in that way, the signal of FNUCs was no longer detected and the 5FU signal was increased, therefore confirming the previous attribution.

As can be seen from Fig. 2 and 3A, NMR signals are very broad in the living fungus. Moreover, the ^{19}F NMR chemical shift range of the various FNUCt is very small (≈0.5 ppm at pH ≈5.5). NMR resolution is not, therefore, sufficient to allow accurate assignment and quantification in the living fungus. To overcome this, small free metabolites and macromolecule-bound fluorinated metabolites were extracted with PCA from *S1* mycelia submitted to in vivo NMR recording.

Spectra of the cold PCA extracts from 3- and 8-day-old cultures showed the resonances of FNUCt (−88.90 ppm), FNUCs (−89.96 ppm), and 5FU (−93.36 ppm). Two small signals in the resonance area of FNUCt (−89.05 and −89.10 ppm) were also detected in the cold PCA extract from the 4.5-day-old cultures. As in living *S1* mycelia, the signal of FBAL (−112.64 ppm) was present only in the spectra of cold PCA extracts from 3- and 4.5-day-old cultures (Fig. 3C). The main signal of FNUCt was assigned to FUTP by adding this standard to the 3-day-old cold PCA extract. As it has been observed in *Candida* strains (26), the signal of FNUCs came from a FNUCt conversion during the NMR recording of the living fungus, since it was undetected in a cold PCA extract prepared immediately at the end of the culture when the fungus was not submitted to unfavorable survival conditions of NMR recording. It could therefore be attributed to FUrd.

Two signals (−89.52 and −89.81 ppm) were observed in the spectra of hot PCA extracts (Fig. 3D). These signals were different from those observed in the cold PCA extracts,

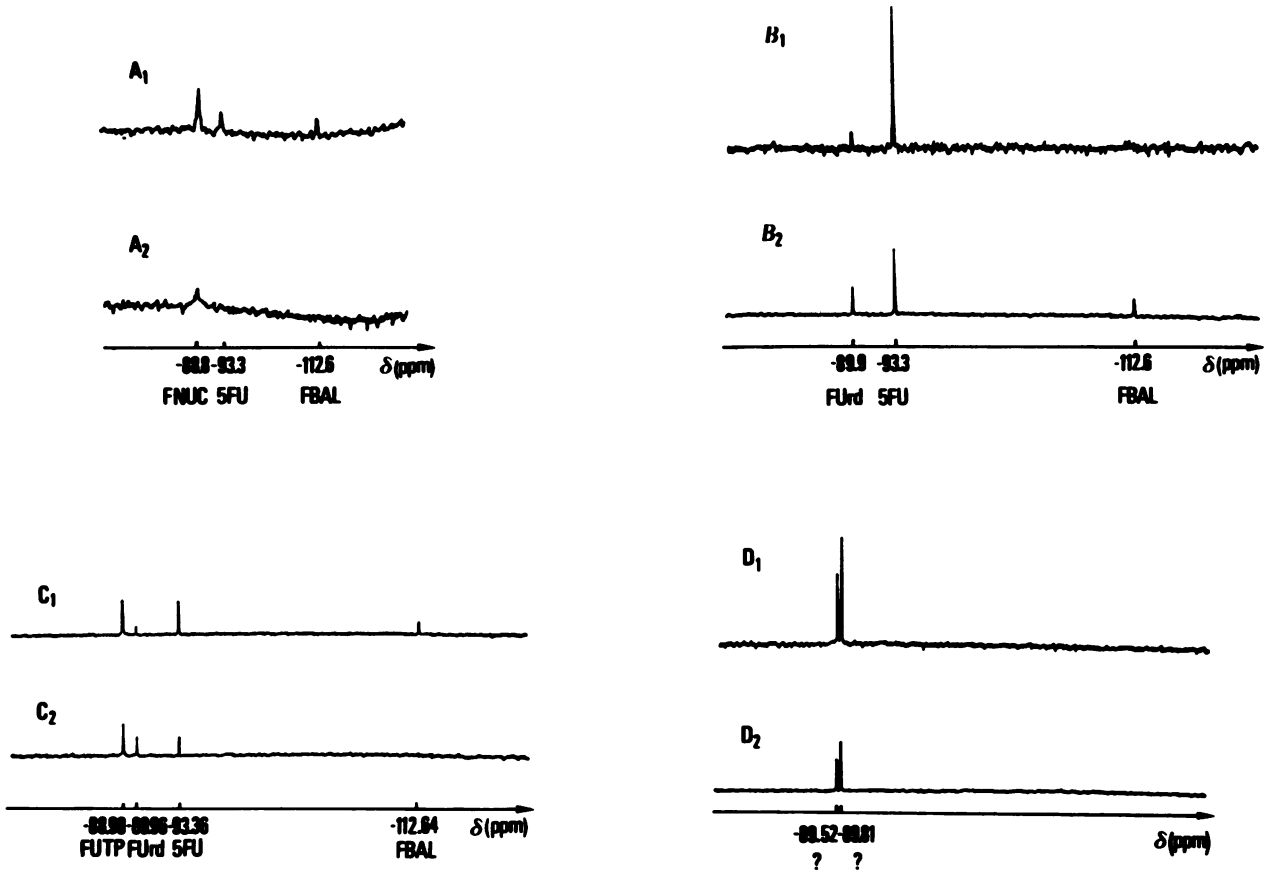

FIG. 3. A, ^{19}F NMR spectra of living mycelia of *S1* strain after 3 (A_1) and 8 (A_2) days on MSA containing 76.9 μmoles of 5FU per liter; B, corresponding agar media; C, corresponding cold PCA extracts (C_1, pH = 5.34; C_2, pH = 5.37); D, corresponding hot PCA extracts (D_1, pH = 4.75; D_2, pH = 5.07). ?, Unidentified compounds.

and none of them corresponded to FdUMP, as was demonstrated by adding this standard to a hot PCA extract; indeed, the signal of FdUMP was located at −89.42 ppm. From the values of their chemical shifts, they probably correspond to phosphorylated 5FU derivatives, but they remain unidentified at present.

The intracellular content of 5FU and its metabolites in *S1* mycelium was tentatively evaluated by using the data obtained from PCA extracts (Fig. 4). The sum of the concentrations of all detected intracellular fluorinated compounds decreased from 244 to 143 nm per g of mycelium between days 3 and 8 of culture. These quantities correspond, respectively, to 2.5 and 1.7% of the amount of 5FU initially present in the medium which supported the growth of the mycelium. The decrease in the level of fluorinated intracellular compounds relative to mycelial weight more likely reflects the accumulation of nonfluorinated structural components responsible for weight increase during growth rather than a true reduction in the absolute amounts of fluorocompounds. These amounts are low compared with the quantities of fluorinated compounds found in yeasts (26). The 5FU concentration per gram of mycelium was reduced by 50% between days 3 and 4 of growth and then stayed at about 22 nm per gram until 8 days. 5FU represented 20% of the intracellular concentration of fluorinated compounds for day 3 of growth, then 15% at 4.5 and 8 days. The FNUCt plus FNUCs concentration was reduced by ≈35% between days 3 and 4 of growth and then stayed at about 115 nm per gram. Figure 4 shows that FNUCt plus FNUCs were the major metabolites of the drug. They made up ≈75% of the intracellular concentration of fluorinated compounds at 3 and 4.5 days and 85% at day 8. Only a low level of FBAL was present at 3 and 4.5 days (≈15 nm per gram of mycelium). This catabolite represented ≈8% of the cellular fluorinated

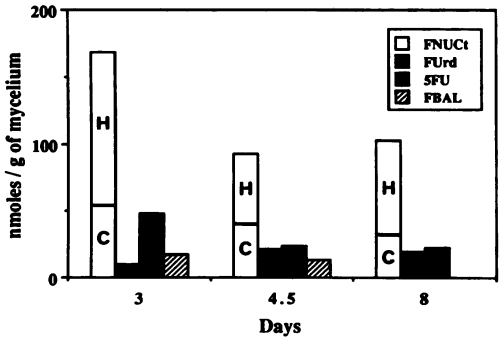

FIG. 4. Intracellular concentration of 5FU metabolites in the *S1* strain of *N. haematococca* from a ^{19}F NMR study of hot and cold PCA extracts at 3, 4.5, and 8 days of growth on MSA containing 76.9 μmoles of 5FU per liter. The term FNUCt represents here the two unknown fluorinated compounds found in the hot PCA extracts and FUTP (or FUTP plus FNUCt signals at −89.05 and −89.10 ppm for 4.5 days) found in the cold PCA extracts. H, Hot PCA extract; C, cold PCA extract.

content. These data provide evidence that at least two 5FU metabolic pathways are operational in the *S1* wild-type strain of *N. haematococca*. Anabolism to phosphorylated derivatives appears to be the main pathway, whereas catabolism to FBAL is only of minor importance.

(iii) ^{19}F NMR study of the *furA3* strain grown on MSA containing 76.9 μM 5FU. No resonance was obtained from 3-, 4-, or 7-day-old *furA3* mycelia grown on MSA medium containing 76.9 μmoles of 5FU per liter and submitted to in vivo NMR measurements. Only the signal of 5FU could be observed in the corresponding media. 5FU was always found in the cold PCA extracts. FBAL was also detected in the cold PCA extract from the 7-day-old culture. A very low level of fluoride anion (F^-) that could not be accurately quantified was observed in the hot PCA extracts. The occurrence of F^- was thought more likely to result from artifactual defluorination of 5FU during the preparation of the PCA extracts, rather than from a metabolic reaction.

The intracellular concentration of 5FU and metabolites was about 20 nm per gram of mycelium, i.e., ≈0.5% of the amount of 5FU initially present in culture media. Therefore, 5FU uptake appeared to be decreased in *furA3* mycelium compared with *S1*. It is known that 5FU and uracil share the same transport system (25). Preliminary measurements of the uptake of [2-^{14}C]uracil by the two strains of *N. haematococca* were done by using the methods mentioned by Buxton and Radford (3). When 10^6 5-hour-old germinating microconidia were suspended in 2 ml of liquid medium containing 0.1 μmole of [2-^{14}C]uracil (55 mCi/mmole), about 4.6 and 1.6% of the radioactivity were taken up within the first 3 min by the *S1* and *furA3* cells, respectively. Buxton and Radford (3) also found that *N. crassa* mutants lacking UPRTase showed a reduced rate of uracil uptake. The reduced rate of 5FU transport in *furA3* cells may explain why FBAL was observed only in the 7-day samples. After 7 days of growth, FBAL represented ≈40% of the intracellular fluorinated content (≈8 nm per gram of mycelium, i.e., half of the amount measured in the wild type).

The above results show that *furA3* is impaired in 5FU anabolism as a consequence of an UPRTase deficiency. The phenotype of this mutant is characterized by an unaltered growth rate and a lack of pigment synthesis in the presence of 5FU (19). These data indicate that 5FU likely exerts its stimulatory effect on the pigmentation of the wild type through conversion to a phosphorylated anabolite.

These experiments also demonstrate that ^{19}F NMR spectroscopy is a valuable tool for studying the metabolism of fluorine-containing compounds in both living hyphae and aqueous extracts of filamentous fungi. In this particular case, 5FU anabolites were clearly distinguished from unmetabolized 5FU and products of its catabolism. This approach may represent an alternative to the biochemical methods involving enzyme analysis, chromatographic techniques, and use of radioactive tracers.

LITERATURE CITED

1. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
2. **Buxton, F. P., and A. Radford.** 1982. Isolation and mapping of 5-fluoropyrimidine resistant mutants of *Neurospora crassa*. Mol. Gen. Genet. **185**:129–131.
3. **Buxton, F. P., and A. Radford.** 1982. Partial characterization of 5-fluoropyrimidine resistant mutants of *Neurospora crassa*. Mol. Gen. Genet. **185**:132–135.
4. **DiVito, M., F. Podo, A. Torosantucci, G. Carpinelli, W. L. Whelan, D. Kerridge, and A. Cassone.** 1986. A ^{19}F nuclear magnetic resonance study of uptake and metabolism of 5-fluorocytosine in susceptible and resistant strains of *Candida albicans*. Antimicrob. Agents Chemother. **29**:303–308.
5. **Dreyer, R., and E. C. Cadman.** 1981. Use of periodate and methylamine for the quantitation of intracellular 5-fluoro-2′-deoxyuridine-5′-monophosphate by high performance liquid chromatography. J. Chromatogr. **219**:273–284.
6. **Galun, E., and J. Gressel.** 1966. Morphogenesis in *Trichoderma*: suppression of photoinduction by 5-fluorouracil. Science **151**: 696–698.
7. **Garrett, C., and D. V. Santi.** 1979. A rapid and sensitive high pressure liquid chromatography assay for deoxyribonucleoside triphosphates in cell extracts. Anal. Biochem. **99**:268–273.
8. **Gochin, M., T. L. James, and R. H. Shafer.** 1984. *In vivo* ^{19}F NMR of 5-fluorouracil incorporation into RNA and metabolites in *Escherichia coli* cells. Biochim. Biophys. Acta **804**:118–124.
9. **Heidelberger, C., P. V. Danenberg, and R. G. Moran.** 1983. Fluorinated pyrimidines and their nucleosides. Adv. Enzymol. Relat. Areas Mol. Biol. **54**:58–119.
10. **Horowitz, J., and V. Kohlmeier.** 1967. Formation of active β-galactosidase by *Escherichia coli* treated with 5-fluorouracil. Biochim. Biophys. Acta **142**:208–218.
11. **Jones, G. E.** 1984. 6-Azauracil resistant variants of cultured plant cells lack uracil phosphoribosyltransferase activity. Plant Physiol. **75**:161–165.
12. **Jund, R., and F. Lacroute.** 1970. Genetic and physiological aspects of resistance to 5-fluoropyrimidines in *Saccharomyces cerevisiae*. J. Bacteriol. **102**:607–615.
13. **Keniry, M., C. Benz, R. M. Shafer, and T. L. James.** 1986. Noninvasive spectroscopic analysis of fluoropyrimidine metabolism in cultured tumor cells. Cancer Res. **46**:1754–1758.
14. **Lindenmayer, A., and H. F. Schoen.** 1967. Selective effects of purine and pyrimidine analogues and of respiratory inhibitors on perithecial development and branching in *Sordaria*. Plant Physiol. **42**:1059–1070.
15. **Malet-Martino, M. C., F. Faure, J. P. Vialaneix, C. Palevody, E. Hollande, and R. Martino.** 1986. Noninvasine fluorine-19 NMR study of fluoropyrimidine metabolism in cell cultures of human pancreatic and colon adenocarcinoma. Cancer Chemother. Pharmacol. **18**:5–10.
16. **O'Byrne-Ring, N., and E. Duke.** 1980. Biochemical and genetic basis of the response to 5-fluorouracil in *Drosophila melanogaster*. Biochem. Genet. **18**:717–726.
17. **Palmer, L. M., C. Scazzocchio, and D. J. Cove.** 1975. Pyrimidine biosynthesis in *Aspergillus nidulans*. Isolation and characterization of mutants resistant to fluoropyrimidines. Mol. Gen. Genet. **140**:165–173.
18. **Parente, A., R. Arking, and K. Kalata.** 1980. Effect of DNA inhibitors upon DNA synthesis and development of *Drosophila* embryos. J. Exp. Zool. **212**:195–204.
19. **Parisot, D.** 1988. 5-Fluorouracil stimulation of naphthoquinone synthesis in *Nectria haematococca* (Berk. and Br.) Wr. Exp. Mycol. **12**:35–46.
20. **Parisot, D., M. Maugin, and C. Gerlinger.** 1984. Genes controlling pigmentation in *Nectria haematococca*. J. Gen. Microbiol. **130**:1543–1555.
21. **Polak, A., and H. J. Scholer.** 1980. Mode of action of 5-fluorocytosine. Rev. Inst. Pasteur Lyon **13**:233–244.
22. **Restivo, F. M., and F. Tassi.** 1986. Effect of 5-fluorouracil on growth and morphogenesis of tissue cultures of *Nicotiana sylvestris*. Plant Cell Physiol. **27**:785–790.
23. **Schmidt, F. R., P. A. Lemke, and K. Esser.** 1986. Viral influences on aflatoxin formation by *Aspergillus flavus*. Appl. Microbiol. Biotechnol. **24**:248–252.
24. **Teal, R., and D. P. Nagle, Jr.** 1986. Effects of 5-fluorouracil on growth and methanogenesis in *Methanobacterium thermoautotrophicum* (Marburg). Curr. Microbiol. **14**:227–230.
25. **Valeriote, F., and G. Santelli.** 1984. 5-Florouracil (FUra). Phar-

macol. Ther. **24**:107–132.
26. **Vialaneix, J. P., N. Chouini, M. C. Malet-Martino, R. Martino, G. Michel, and J. P. Lepargneur.** 1986. Noninvasive and quantitative ^{19}F nuclear magnetic resonance study of flucytosine metabolism in *Candida* strains. Antimicrob. Agents Chemother. **30**:756–762.
27. **Wain, W. M., and W. D. Staatz.** 1973. Rates of synthesis of ribosomal protein and total ribonucleic acid through the cell cycle of the fission yeast *Schizosaccharomyces pombe*. Exp. Cell Res. **81**:269–278.
28. **Waldorf, A. R., and A. Polak.** 1986. Mechanisms of action of 5-fluorocytosine. Antimicrob. Agents Chemother. **23**:79–85.