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Author	河野, 彬(Kono, Akira) 菅田, 節朗(Sugata, Setsuro) 原, 泰寛(Hara, Yasuhiro) 田中, 睦子(Tanaka, Mutsuko) 加留部, 善晴(Karube, Yoshiharu) 松島, 美一(Matsushima, Yoshikazu)
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**Assay for Pyrimidine Nucleoside Phosphorylases in Tissue
Extracts by High-Performance Liquid Chromatography***

Akira KONO,** Setsuro SUGATA, Yasuhiro HARA,** Mutsuko TANAKA,**
Yoshiharu KARUBE,** and Yoshikazu MATSUSHIMA

河野 彬,** 菅田節朗, 原 泰寛,** 田中睦子,**
加留部善晴,** 松島美一

Pyrimidine nucleoside phosphorylases play important roles in the metabolism of nucleic acids and are present in normal and neoplastic tissues. It has been claimed that they are responsible for the catabolism of chemical depot forms of 5-fluorouracil (FU) such as 5'-deoxy-5-fluorouridine (DFUR) and 1-(tetrahydro-2-furanyl)-5-fluorouracil (Tegafur) with real or potential chemotherapeutic usefulness.

A number of assay methods have been proposed for pyrimidine nucleoside phosphorylases. Some are based on the differences in the UV spectra of nucleosides and pyrimidine base in strong alkaline solutions. Most of the assay methods are based on the separation and determination of the phosphorylated products, either sugar or base moieties. Radiolabeled substrates are frequently used in the determination of the bases. Generally, spectrometric methods suffer from the inaccuracies and the separation methods from the tedium.

High-performance liquid chromatography was successfully applied for the determination of pyrimidines formed enzymatically from thymidine, uridine, 5-fluorouridine, and 5'-deoxy-5-fluorouridine. The pyrimidines, after extraction with ethyl acetate, are separated by reversed-phase chromatography on μ -Bondapak C-18/Porasil and determined from the heights of chromatographic peaks with UV detection. The method is simple, sensitive and reliable. The limits of detection are 2.5 pmol, 1.0 pmol, and 2.0 pmol for thymine, uracil, and 5-fluorouracil, respectively.

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** 九州がんセンター