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This Week's Citation Classic

Cohen S S, Flaks J G, Barner H D, Loeb M R & Lichtenstein J. The mode of action of 5-fluorouracil and its derivatives. *Proc. Nat. Acad. Sci. US* **44**:1004-12, 1958. [Depts. Biochem. and Pediat., Univ. Pennsylvania Sch. Med., Philadelphia, PA]

The antitumor agent 5-fluorouracil, and its deoxyribosyl derivative are converted to the deoxyribonucleotide in *E. coli* and provoke thymine deficiency and 'thymineless death.' Fluorodeoxyuridylate, isolated from the bacteria or synthesized enzymatically *in vitro*, is an irreversible inhibitor of the thynidylate synthetase isolated from phage-infected bacteria. [The *SCI*® indicates that this paper has been cited over 495 times since 1961.]

Seymour S. Cohen
Department of
Pharmacological Sciences
State University of New York
Stony Brook, NY 11794

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"When G.R. Wyatt and I discovered 5-hydroxymethylcytosine (HMC) in 1952,[1] my laboratory began to study its biosynthesis. In 1953, Hazel Barner and I found that a thymine deficiency led to the death of growing bacteria,[2] and we suggested that this might explain the antitumor effects of some antifolates. We had also observed a phage-induced synthesis of thymine and HMC in bacteria auxotrophic for thymine. By 1957, joel Flaks and I had found that extracts of phage-infected bacteria contained large amounts of two virus-induced enzymes which made the viral pyrimidines as deoxyribonucleotides.[3] Such extracts provided the most active sources of thymidylate synthetase, permitting a study of inhibitors of this enzyme apparently crucial to DNA synthesis and cell survival. In that year, Charles Heidelberger and Robert Duschinsky had discovered that 5-fluorouracil markedly inhibited tumors in mice,[4] and they asked me to apply our bacterial and phage systems to clarifying the mode of action of the analog on thymine synthesis. I readily accepted their invitation, with the results presented in the abstract. I also suggested to Duschinsky that fluorocytosine might be a selective antifungal agent and was pleased to learn in 1959[5] that fluorocytosine was specifically inhibitory to these organisms.

"Heidelberger has extended many detailed studies with fluorouracil in human cancer and helps to lead a cancer center at USC. Duschinsky has retired to almost full-time skiing in Switzerland. As a result of experiments in 1956 with spongothymidine[6] and our first experiences in cancer chemotherapy, I became interested in the potentialities of the D-arabinosyl nucleosides and problems of chemotherapy in general. Flaks is currently a professor of biochemistry at the University of Pennsylvania. Our other co-workers have raised families and have then returned to the laboratory.

"Although it is now some 20 years since the discovery that fluorouracil provokes 'thymineless death,' the nature of these events is less than crystal clear. Most workers today do believe that antitumor therapy with fluorouracil, as well as agents such as inhibitors of dihydrofolate reductase, e.g., amethopterin, does produce a thymine deficiency. The importance of this effect in chemotherapy has led to increasingly detailed studies of the pure synthetase and reductase and their inhibition. The primary sequences of these key enzymes determined by an infecting parasite and host should be quite different. A thorough comparison of the parasite-and host-determined enzymes may then provide a rational approach to the development of a chemotherapy necessary to selectively inhibit or kill an infecting organism."

1. **Wyatt G R & Cohen S S.** The bases of the nucleic acids of some bacterial and animal viruses: the occurrence of 5-hydroxymethylcytosine. *Biochemical J.* **55**:774-82, 1953.
2. **Barner H D & Cohen S S.** The induction of thymine synthesis by T2 infection of a thymine requiring mutant of *Escherichia coli. J. Bacteriology* **68**:80-8, 1954.
3. **Flanks J G & Cohen S S.** The enzymic synthesis of 5-hydroxymethyldeoxycytidylic acid. *Biochim. Biophys. Acta* **25**:667-8, 1957.
4. **Heidelberger C, Chaudhuri N K, Danneberg P, Mooren D, Griesbach L, Duschinsky R, Schnitzer R J, Pleven E & Scheimer J.** Pluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature* **179**:663-6, 1957.
5. **Duschinsky R.** Personal communication.
6. **Cohen S S & Barner H D.** Studies on the induction of thymine deficiency and on the effects of thymine and thymidine analogues in *Escherichia coli. J. Bacteriology* **71**:588-97, 1956.