adrenal medulla and the close association with unmyelinated axons are certainly consistent with the physiological hypothesis requiring a peripheral store of noradrenaline.

Search for these cells in thin sections of human skin prepared for electron microscopy may be a time-consuming and disappointing task. Thus further observations may be dependent on incidental recognition during the course of other studies.

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Uracil: Failure To Restore DNA Synthesis While Relieving 5-Fluorouracil-Induced Inhibition

Abstract. *Growth inhibition of* Bacillus cereus *by 5-fluorouracil is abolished by the addition of uracil without the inhibition of DNA synthesis being relieved. The growth rate, protein, and RNA content of cells grown in the presence of 5-fluorouracil and uracil are unaffected until the cells contain only about 25 percent of their normal DNA content.*

The growth-inhibitory agent 5-fluorouracil (*1*) has been shown to inhibit DNA synthesis in Ehrlich ascites cells (*2*). The active form of the analog is believed to be 5-fluoro-2′-deoxyuridine-5′-monophosphate which acts as a competitive inhibitor of thymidylate synthetase (*3*). The conversion of the drug to the active metabolite and its effect on DNA synthesis in bacterial cells results in growth inhibition and "thymineless death" (*4*). In this report we suggest that the growth-inhibitory action of 5-fluorouracil can be dissociated from the drug's inhibition of DNA synthesis in *Bacillus cereus* by the addition of uracil together with 5-fluorouracil.

Bacillus cereus 569 H was grown at 37°C in the presence or absence of 5-fluorouracil (20 μg/ml; 0.16 μmole/ml). Growth was measured by changes in optical density (OD) in a Bausch and Lomb Spectronic colorimeter at 540 mμ. At zero time the drug was added to exponentially growing cells at OD_{540} of 0.3 (equivalent to 0.3 mg dry weight of bacteria per milliliter of culture) together with varying amounts of uracil. In some experiments radioactive nucleic acid precursors were also present. When the cells had grown to OD_{540} of 0.6, the appropriate analysis was performed. Total cell counts were determined by counting the cells, stained with methylene blue, in a Levy counting chamber. Viable counts were determined by pouring a portion of the cell suspension onto nutrient agar plates, incubating overnight at 37°C, and counting the resulting colonies. For control cells the increase in optical density at 540 mμ served as a true indicator of cell division and growth because the cell number at OD_{540} 0.6 was approximately double that at OD_{540} 0.3. In certain experiments growth and DNA synthesis were measured in the presence or absence of only 0.1 μg/ml of 5-fluorouracil added at OD_{540} of 0.02 and the cells grown to 0.64.

The uptake of 5-fluorouracil-2-C^{14} into the total nucleic acid fraction and the incorporation of labeled purines into DNA was measured by the membrane filtration procedure (*5*). Total DNA content was measured spectrophotometrically at 260 mμ in a Beckman DU spectrophotometer after removal of RNA by KOH, and by the Burton method (*6*) for measuring deoxyribose from DNA purine deoxyribosides.

Table 1 shows the time required by the cell suspensions to double in turbidity from an initial OD_{540} of 0.3 (equivalent to one generation time for control cells), the cellular content of radioactive 5-fluorouracil and DNA, and the total cell count when grown with various concentrations of uracil, 5-fluorouracil, and mixtures of the two.

5-Fluorouracil produced a marked depression of DNA synthesis. Cells grown during the period of inhibition (OD_{540} 0.3 to 0.6) synthesized only 16 percent of the normal amount of DNA. Added uracil had no effect on the DNA content of the cells. When the ratio of the molar concentration of uracil to that of 5-fluorouracil was equal to or greater than 1, the incorporation of 5-fluorouracil was diminished (but not abolished) and growth was indistinguishable from that of the control cells. The DNA content, however, was essentially unchanged from that of the 5-fluorouracil cultures and, even at a uracil to 5-fluorouracil ratio of 100, it was still greatly diminished when compared with control cells. The ultraviolet absorption method and the Burton method for DNA provided similar results. The similarity in total cell count between the cultures indicates that the reduction in DNA con-

Table 1. The effect of various concentrations of uracil (U), 5-fluorouracil (5-FU), and mixtures of both on the growth rate, DNA content, and cell count of *Bacillus cereus*.

Culture	U (μg/ml)	5-FU (μg/ml)	U : 5-FU (molar ratio)	Turbidity doubling time (min)	5-Fluorouracil-2-C^{14}		DNA		Cell count × 10^{-9} (per ml)
					Total 5-FU in cell* (μg per mg dry wt)	5-FU in RNA* (μg per mg RNA)	μg per mg dry wt	μg per mg dry wt (% increase)†	
Control				50			21.9	(100)	1.48
Control+ uracil	18			50			22.3	103	1.52
5-Fluoro-uracil		20		180	(100)	(100)	11.2	16	1.28
5-FU + U	1.8	20	0.1	90	94	83			
5-FU + U	18	20	1	55	15	8	10.6	12	
5-FU + U	180	20	10	55	2	1	11.0	15	1.34
5-FU + U	1800	20	100	55	0.7	0.2	11.6	20	

* Values expressed as a percentage of 5-FU culture. Actual values are 8.02 μg 5-FU per milligram of dry weight, and 13.9 μg 5-FU incorporated into RNA per milligram of RNA. † The value at OD_{540} 0.3 (9.1 μg DNA per milligram of dry weight) was subtracted from the DNA content at 0.6 and expressed as a percentage of the control.

Table 2. Incorporation of radioactive nucleic acid precursors into the KOH-insoluble residue (DNA). (5-FU, 5-fluorouracil; U, uracil.)

Precursor	Control (count/min)	5-FU* (20 μg/ml)	5-FU + U* (20 μg/ml + 18 μg/ml)	Control + U* (18 μg/ml)
Adenine-8-C^{14}	(100)	28	14	102
Guanine-2-C^{14}	(100)	35	17	99
Hypoxanthine-8-C^{14}	(100)	21	9	109
Sodium formate-C^{14}	(100)	46	23	103

* Values expressed as a percentage of the control culture. Actual control values ranged from 500 to 30,000 count/min. Each value is the average of two to five determinations.

tent is not caused by a reduction in cell number but represents a real difference in the average DNA content per cell. The viable cell count, however, was reduced after growth in 5-fluorouracil and was not restored to normal by the addition of uracil.

Incorporation of radioactive purine precursors into DNA again confirmed the reduced DNA synthesis in the presence of 5-fluorouracil (Table 2). When uracil was added at the same time, at a uracil to 5-fluorouracil ratio of 1, results were produced which implied an even greater depression of uptake into DNA than did 5-fluorouracil alone. It must be realized, however, that the isotopic method of analysis measured only radioactivity in newly formed DNA whereas the total DNA was analyzed by the Burton method. The DNA synthesized before the addition of 5-fluorouracil, or 5-fluorouracil plus uracil was stable during subsequent growth, as measured by incubation studies with isotopes (*7*). Protein and RNA synthesis, measured colorimetrically and by isotope incorporation, were not affected by the inhibition of growth and of DNA synthesis produced by 5-fluorouracil. Other experiments (*8*) have shown that thymidine, if added with the drug at zero time and every 15 minutes thereafter, can maintain the DNA content at a control level but can not prevent the inhibition of growth.

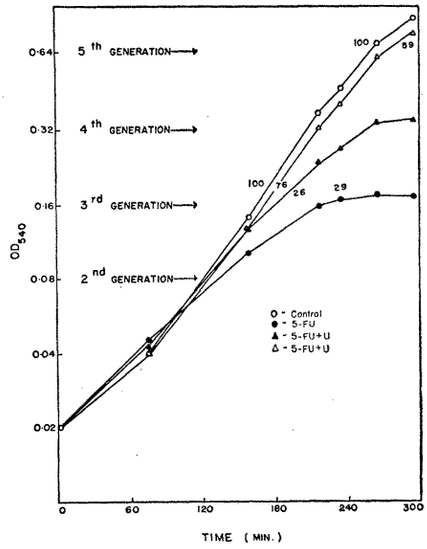

Fig. 1. Growth of *B. cereus*. Solid circles, 5-fluorouracil (5-FU) (0.1 μg/ml); solid triangles, 5-FU (0.1 μg/ml) plus uracil (0.9 μg/ml); open triangles, 5-FU (0.1 μg/ml) plus uracil (9 μg/ml). Numbers refer to DNA content, expressed as a percentage of the control value which is equal to 100.

At a drug concentration of 0.1 μg/ml (initial OD_{540} 0.02) there was a complete cessation of growth after three generations (Fig. 1). At a uracil to 5-fluorouracil ratio of 10, growth was inhibited (after three generations) when the DNA content, as measured by adenine-8-C^{14} incorporation, was reduced to 26 percent of the control, but did not terminate until after the fourth generation. A ratio of 100 was able to maintain a normal rate of growth for five generations although the cells contained only 60 percent of the DNA content of the controls at a comparable turbidity.

These results indicate that the inhibitory action of 5-fluorouracil on DNA synthesis is not reflected in the growth rate until the DNA content is diluted by growth, in this case after three generations, to a minimum quantity equal to 20 to 30 percent of the control value. Thus, about 75 percent of the normal DNA content of *B. cereus* may not be essential for growth, protein, or RNA synthesis. These results are perhaps related to those reported by Allfrey and Mirsky (*9*) who reported that up to 80 percent of the DNA in thymus nuclei could be removed without impairing protein or RNA synthesis. They concluded that most of the DNA was inactive and existed in a repressed state.

In *B. cereus* the presence of excess, inactive DNA may be associated with the multinucleate nature of the cell. Actively growing cells may contain as many as four separate chromatin bodies containing deoxyribonucleoprotein (*10*).

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Hemoglobin Synthesis in Marrow Cell Culture: The Effect of Rat Plasma on Rat Cells

Abstract. *In cultures of rat marrow cells essentially all of the labeled iron which was incorporated into heme was shown to be in hemoglobin. Iron bound to rat plasma was incorporated into hemoglobin to a greater extent than was iron bound to fetal calf serum. Incorporation of iron bound to rat plasma was greater when some fetal calf serum was present than when rat plasma provided the total protein. These data suggest preferential use of homologous iron-binding proteins of the plasma.*

In a recent paper (*1*) we showed that continued heme synthesis by cultures of bone marrow cells from the rat for periods longer than about 12 hours was dependent upon the presence of erythropoietin in the culture medium. The utility of marrow cell cultures for the assay of erythropoietin, and for the investigation of the chemical mode of action of the hormone as a model for the study of differentiation, has been the subject of further experimentation with this system.

Our earlier paper contained indirect evidence that a simple procedure in which the extraction of heme from an acidified lysate of marrow cells into butanone (*2*) followed by the measurement of heme radioactivity is adequate for the determination of hemoglobin synthesis. Since one well-defined end product of stem-cell differentiation is hemoglobin, it was important to demonstrate directly that the as-