determined by both the duration of drug exposure and the proliferative state of the cells.

These findings are reminiscent of similar effects encountered by Neuhard & Munch-Petersen (1966) and Neuhard & Thomassen (1971) when *Escherichia coli* cells were deprived of thymine. These authors have also studied the changes in turnover rates of deoxynucleotide pools in *E. coli* during thymine starvation: they found that thymine deprivation influenced differentially the rates of deoxynucleotide catabolism and anabolism.

The relative proportions of dATP and dTTP are important in regulating the activity of ribonucleotide reductase in both bacterial and mammalian cells (Reichard, 1968). Further, methotrexate inhibits both purine and pyrimidine biosynthesis, and its toxicity is enhanced by the presence of adenosine (Borsa & Whitmore, 1969). There are two possible interpretations of these findings: (1) for maximum cytotoxicity the inhibitory effects of methotrexate should be focused on one (critical) pathway, namely thymidylate biosynthesis; (2) exogenous adenosine would generate high pool concentrations of dATP, thus magnifying the inhibitory allosteric effects (in conjunction with the low dTTP pool) on ribonucleotide reductase.

Experiments designed to elucidate these possibilities will be discussed.

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A Factor Responsible for Thymineless Death in *Bacillus subtilis*

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Although thymineless death is a well-established phenomenon, the mechanism whereby the cells lose their viability remains obscure. Much evidence has suggested that death occurs as a result of some alteration or damage to the bacterial DNA or DNA-synthesizing systems (Schaiberger *et al.*, 1967).

We have investigated thymineless death in *Bacillus subtilis*. Initial studies demonstrated that the rate of loss of viability was dependent on the cell concentration, suggesting that the concentration of a factor in

the culture was critical in determining the rate of cell death; indeed, when the supernatant fluid of a starved culture was added back to a newly deprived culture, the rate of cell death increased. This situation is analogous to that reported by Barlati & Majerfeld (1970) for tryptophanless death in *B. subtilis*. The supernatant fluid also has a marked inhibitory effect on the ability of cells undergoing thymineless death to incorporate [6-³H]thymidine into trichloroacetic acid-insoluble material.

The killing activity is not produced continuously during incubation in thymineless medium, but is present in maximum amounts at zero time. This implies that the active constituent is present in normally growing cells and that the process of re-suspension of the cells in thymineless medium releases the factor. Cell washes prepared throughout the normal growth cycle showed that the activity was maximal in early exponential phase, and preliminary evidence indicates a correlation between exonuclease and lethal activities.

The results will be discussed in terms of a mechanism for thymineless death, involving disruption of the normal balance between nucleolytic and polymerase activities.

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A Comparison of the Utilization of Thymine and Thymidine as Precursors for Deoxyribonucleic Acid Synthesis in *Bacillus subtilis*, and their Specific Inhibition by 6-(p-Hydroxyphenylazo)uracil and Caffeine

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A study has been made of the efficiency of incorporation of [6-³H]thymine and [6-³H]thymidine into trichloroacetic acid-soluble and trichloroacetic acid-insoluble fractions of cells of *Bacillus subtilis*. Cells were pulse-labelled for 4 min, and the fate of the absorbed radioactivity was investigated during subsequent incubation for 10 min after the addition of unlabelled thymine or thymidine. It was found that [6-³H]thymidine is taken up into cells with a tenfold greater efficiency than thymine, although, once within the cells, the relative amounts of radioactivity transferred into the acid-insoluble fractions are approximately equivalent. The nature of the acid-soluble fraction has been investigated by polyethylimine-cellulose chromatography, and results suggest that dTTP is produced solely from thymine.

To investigate this phenomenon further, we have studied the effect of 6-(*p*-hydroxyphenylazo)uracil, a specific inhibitor of DNA replication (Brown, 1971), and caffeine, an inhibitor of DNA repair (Harm, 1967). Evidence will be presented indicating that 6-(*p*-hydroxyphenylazo)uracil strongly inhibits thymidine utilization although it has only a minimal effect on thymine utilization. Caffeine, on the other hand, exerts a preferential effect on thymine utilization.

Further, comparison of the efficiency of utilization of [$6\text{-}^3\text{H}$]thymine and [$6\text{-}^3\text{H}$]thymidine after u.v. irradiation of cells indicated that, in this case, thymine is used preferentially. This observation is in agreement with the result obtained by Billen *et al.* (1971), that thymine utilization by freeze-treated cells is unaffected by irradiation whereas thymidine utilization is greatly diminished. It also supports the concept of these authors that thymine and thymidine have different pathways for DNA synthesis. More than that, however, the present results suggest that both 'repair' and 'replicative' DNA synthesis are occurring simultaneously during normal growth in *B. subtilis*.

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The Heterogeneity of Chromatin Non-Histone Proteins

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There is now considerable evidence to indicate that the acidic non-histone proteins of chromatin may be associated with the control of DNA transcription (see review by MacGillivray *et al.*, 1972). Hence, if these proteins possess regulatory or specificity-determining activities, it would be reasonable to expect that some of the components should be cell-specific. In fact a number of studies indicate that this may be so (see, e.g., Kostraba & Wang, 1970; Platz *et al.*, 1970; Teng *et al.*, 1971). However, interpretation of such findings is often difficult owing to preparative methods yielding incomplete extraction or to electrophoretic conditions producing aggregation effects.

We have examined this question by fractionating salt-urea-dissociated chromatins on hydroxyapatite so that the non-histone proteins were obtained in good yield and high purity (MacGillivray *et al.*, 1971). The proteins were then subjected to polyacrylamide-gel electrophoresis in a sodium dodecyl sulphate double-gel system employing discontinuous buffers. The non-

histone proteins were found to be very heterogeneous but similar when isolated from a number of mouse tissues. The extent of this heterogeneity appeared to depend on the method of isolation of cell nuclei, e.g. proteins from 'sucrose' and 'citric acid' nuclei were predominantly of high molecular weight, in contrast with the low-molecular-weight polypeptides obtained from 'double-detergent' nuclei (Penman, 1966). With the exception of brain, the removal of 'cytoplasmic' (Johns & Forrester, 1968) and 'residual' (Wang, 1967) proteins from chromatin also yielded non-histone proteins of low molecular weight. Thus the acidic proteins that remain tightly bound to DNA in chromatin appear to be of the same size as histones.

Comparison of non-histone proteins obtained from 'sucrose' nuclei of several bovine and mouse tissues indicated quantitative rather than qualitative differences, thus confirming the 'limited heterogeneity' of these chromatin proteins (Elgin & Bonner, 1970). In contrast, nucleated erythrocytes or calf thymus chromatins, which contain low amounts of acidic proteins, appear to possess non-histone proteins predominantly of low-molecular-weight species. Since the non-histone proteins are very similar in many chromatins, we are currently investigating their tissue characteristics by protein phosphorylation patterns and template studies *in vitro* (see Rickwood *et al.*, 1972).

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Studies on the Phosphorylation of Chromatin Non-Histone Proteins and their Effect on Deoxyribonucleic Acid Transcription

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The major non-histone proteins have been isolated from chromatin on hydroxyapatite columns (MacGillivray *et al.*, 1971). Sodium dodecyl sulphate-