rotenone or carbonyl cyanide *m*-chlorophenylhydrazine. In the latter case a temporary alkalinization occurred, owing to NADH oxidation, with a $\Delta\text{pH}/2e$ value of -1.0 . Correction of the $\rightarrow\text{H}^+/2e$ values for the non-proton-translocating rotenone-insensitive pathway of quinone reduction by NADH gave calculated values for the proton-translocating rotenone-sensitive pathway of 1.7 – 2.0 . It is concluded that, if the respiratory chain is organized into three proton-translocating loops, then only one of these loops exists between NADH and ubiquinone. The identity of the postulated hydrogen carrier preceding cytochrome *c* remains unknown, unless it be one of the multiple forms of cytochrome *b*.

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Ornithine Decarboxylase Activity during Kidney Growth

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It has been suggested that increased rates of polyamine biosynthesis are associated with rapid tissue growth (Jänne, 1967; Russell & Snyder, 1968; Raina & Jänne, 1970; Snyder & Russell, 1970). The rise in ornithine decarboxylase activity occurring during liver regeneration is in fact one of the earliest and most dramatic responses to partial hepatectomy (Schrock *et al.*, 1970). We have therefore examined ornithine decarboxylase activity (measured by a modification of the $^{14}\text{CO}_2$ absorption method of Russell & Snyder, 1968) in rat kidney and tried to relate it to the hypertrophy that takes place in the remaining kidney after unilateral nephrectomy. Normal rat kidney has a variable content of ornithine decarboxylase about 10–100-fold higher than that of rat liver, spleen, pancreas, heart, brain or uterus. Prostate is the only other tissue with comparable activity. The enzyme activity in the kidney is equally high in male and female rats and is not significantly affected by orchidectomy, ovariectomy or adrenalectomy, or (in the male) by injection of testosterone (25 mg/kg) or oestradiol (2.5 mg/kg). Unilateral nephrectomy produces a sixfold increase in enzyme activity in the remaining kidney 4 h after the operation, but a comparable though slightly smaller increase can be produced by sham operation or by partial hepatectomy. Thereafter the activity decreases, until 12 h after the operation it is at the normal pre-operative value (in unilaterally nephrect-

omized rats) or below it (in sham-operated rats). This increase can be prevented if the animal is maintained under continual Nembutal anaesthesia throughout the 4 h post-operative period or is treated with metyrapone (125 mg/kg) (a drug that inhibits 11β -hydroxylation of steroids). If normal rats are injected with dexamethasone phosphate (0.25 mg/kg) (a synthetic glucocorticoid) their kidneys 4 h later show an increase in the enzyme comparable with that produced by unilateral nephrectomy. Deoxycorticosterone acetate, a mineralocorticoid, has no effect at doses up to 2.5 mg/kg body wt. It seems likely therefore that the increased enzyme activity in the remaining kidney after unilateral nephrectomy is partially a non-specific response to the stress of the operation.

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Changes in the Pyrimidine Deoxynucleoside Triphosphate Pools of L5178Y Cells after their Exposure to Methotrexate and 5-Fluorouracil

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The pools of deoxynucleoside triphosphate in mouse L5178Y lymphoma cells in culture have been measured with a highly sensitive assay utilizing DNA polymerase (Solter & Handschumacher, 1969; Lindberg & Skoog, 1970). Contrary to the finding by Baumunk & Friedman (1971) in HeLa cells, we have observed a fall in the dTTP content of L5178Y cells after treatment with either methotrexate or 5-fluorouracil (at concentrations that inhibit the uptake of $[^3\text{H}]\text{dUMP}$ into DNA). This finding supports the hypothesis that methotrexate and 5-fluorouracil inhibit dTMP syntheses. An elevation in the concentration of the other pyrimidine deoxynucleoside triphosphate, dCTP, occurs subsequent to the fall in that of dTTP. No changes were seen in the concentrations of the purine deoxyribonucleoside triphosphates, dATP and dGTP. The extent of these changes in a heterogeneous population of cells was

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.