TABLE II. Latent Period and Types of Neoplasm.

| Dose of $P^{32}$ ($\mu$c/g body wt) | No. of rats with neoplasm | Latent period (mo) Range | Mean | Types of neoplasm: Osteogenic sarcoma | Types of neoplasm: Squamous cell carcinoma |
|---|---|---|---|---|---|
| 1.0 | 6 | 12 – 25 | 19 | 5 | 1 |
| 2.0 | 4 | 12 – 15 | 14 | 4 | 0 |
| 3.0 | 4 | 8 – 14 | 10.5 | 4 | 0 |
| 3.5 | 8 | 7.5 – 13 | 10 | 5* | 5* |

* Two rats in the 3.5 $\mu$c group had both osteogenic sarcoma and squamous cell carcinoma.

found one instance of osteogenic sarcoma and none of squamous cell carcinoma(3). Ratcliffe(4) reported that about 5% of rats in a Wistar stock colony developed neoplasm and that among the 75 tumor-bearing animals comprising this 5% there was one osteogenic sarcoma and one carcinoma of the skin. The latter arose from the tip of the snout. A very low overall incidence of these two malignant neoplasms has also been described in other strains of the rat(4,5).

In this study the smallest dose of $P^{32}$ which induced tumors was 1 $\mu$c/gram body weight and this corresponds to 70 mc for a 70 kg human, *i.e.*, about 5 times the usual therapeutic dose. Amounts of 0.5 and 0.25 $\mu$c of $P^{32}$ per gram, which approach the average therapeutic dose in man, did not prove carcinogenic. From the experimental standpoint $P^{32}$ can be used as an effective and relatively simple method of inducing malignant neoplasms. The optimum dosage is probably from 1 to 3 $\mu$c per gram body weight. Larger amounts will also be carcinogenic but the early mortality from radiation is potentially greater and the life span of the surviving animals shorter.

*Summary.* 1. Single doses of radioactive phosphorus, administered internally to rats in amounts of from 1 to 3.5 $\mu$c/gram body weight, proved carcinogenic. Two types of malignant neoplasms were produced, *i.e.*, osteogenic sarcoma and carcinoma of the face. 2. The incidence of neoplasms ranged from 22 to 50%. The latent period of tumor development was lengthened with smaller doses of $P^{32}$. 3. Amounts of $P^{32}$ less than 1 $\mu$c/gram body weight failed to induce neoplasms.

1. Koletsky, Simon, Bonte, F. J., and Friedell, H. L., *Cancer Research,* 1950, v10, 129.

2. Brues, A. M., Lisco, H., and Finkel, Miriam, Argonne National Laboratory, MDDC-145.

3. Koletsky, Simon, unpublished data.

4. Ratcliffe, H. L., *Am. J. Path.,* 1940, v16, 237.

5. Curtis, M. R., Bullock, F. D., and Dunning, W. F., *Am. J. Cancer,* 1931, v15, 67.

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## Chemical Inhibition of Cell Division of *Escherichia coli* by Diazo Compounds and Antagonism by Tyrosine. (21071)

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Studies with *Escherichia coli* have shown that certain organic chemicals, such as nitrogen mustards, triethylenemelamine, diazouracil, and other diazo compounds produce the same morphological effects, *i.e.*, the inhibition of multiplication without inhibition of culture growth or mass, as alpha or X-irradiation(1,2) and produce similar biochemical transformations(3). Since 5-diazouracil had proved to be a highly effective cell-division inhibitor and since both 5-aminouracil and

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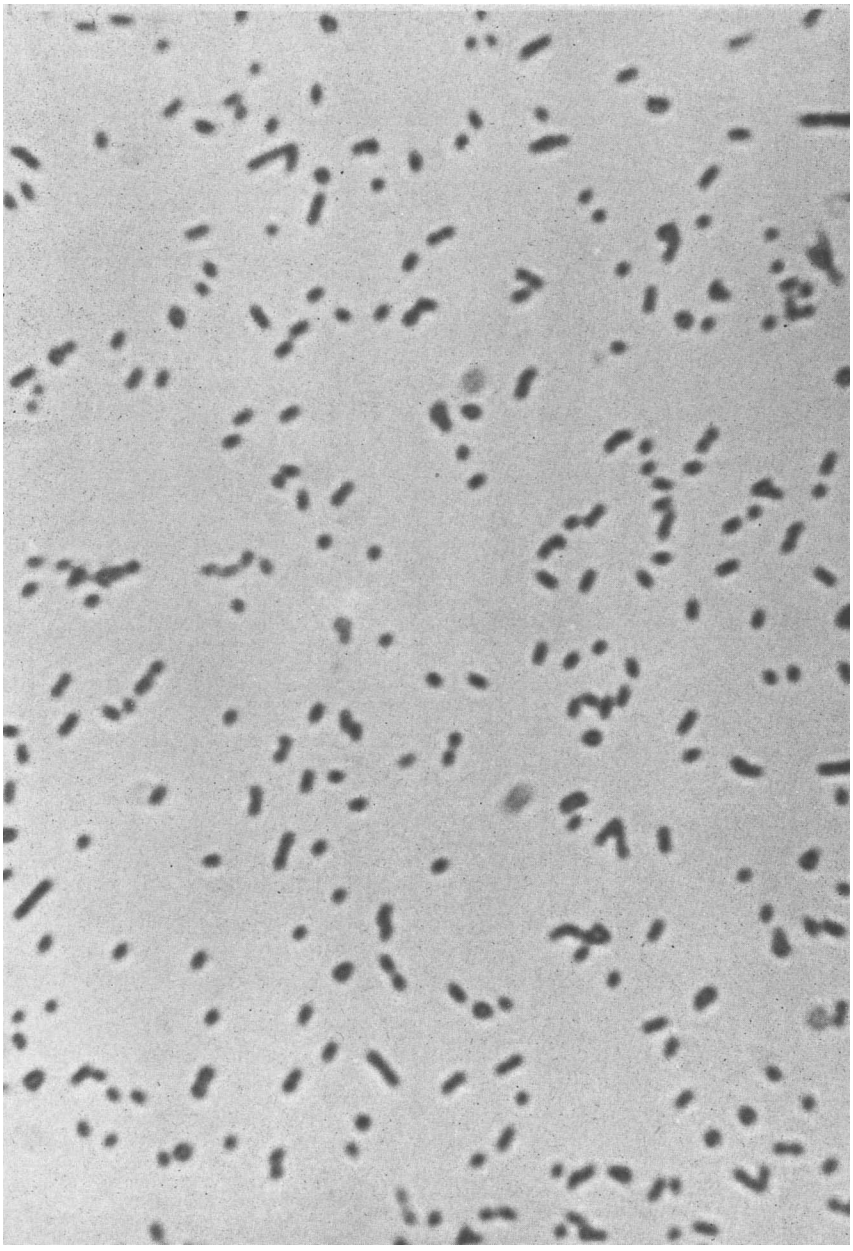

FIG. 1. Control untreated *E. coli* culture in the logarithmic phases of growth and division; cell count, $2 \times 10^9$ cells/ml; culture weight, 0.78 mg/ml (×475).

uracil were inactive compounds, diazouracil was taken as a model for two types of additional studies: a) to test whether the cell division inhibitory activity was due to the diazo group of the molecule; and b) to study the relationship of the inhibition effected by diazouracil to important biochemical metabolites.

The present paper deals with the effects of a variety of aliphatic and aromatic diazo compounds on the division and growth of *E. coli* and with the antagonism of diazouracil division-inhibition by tyrosine.

*Methods.* Growth and testing conditions

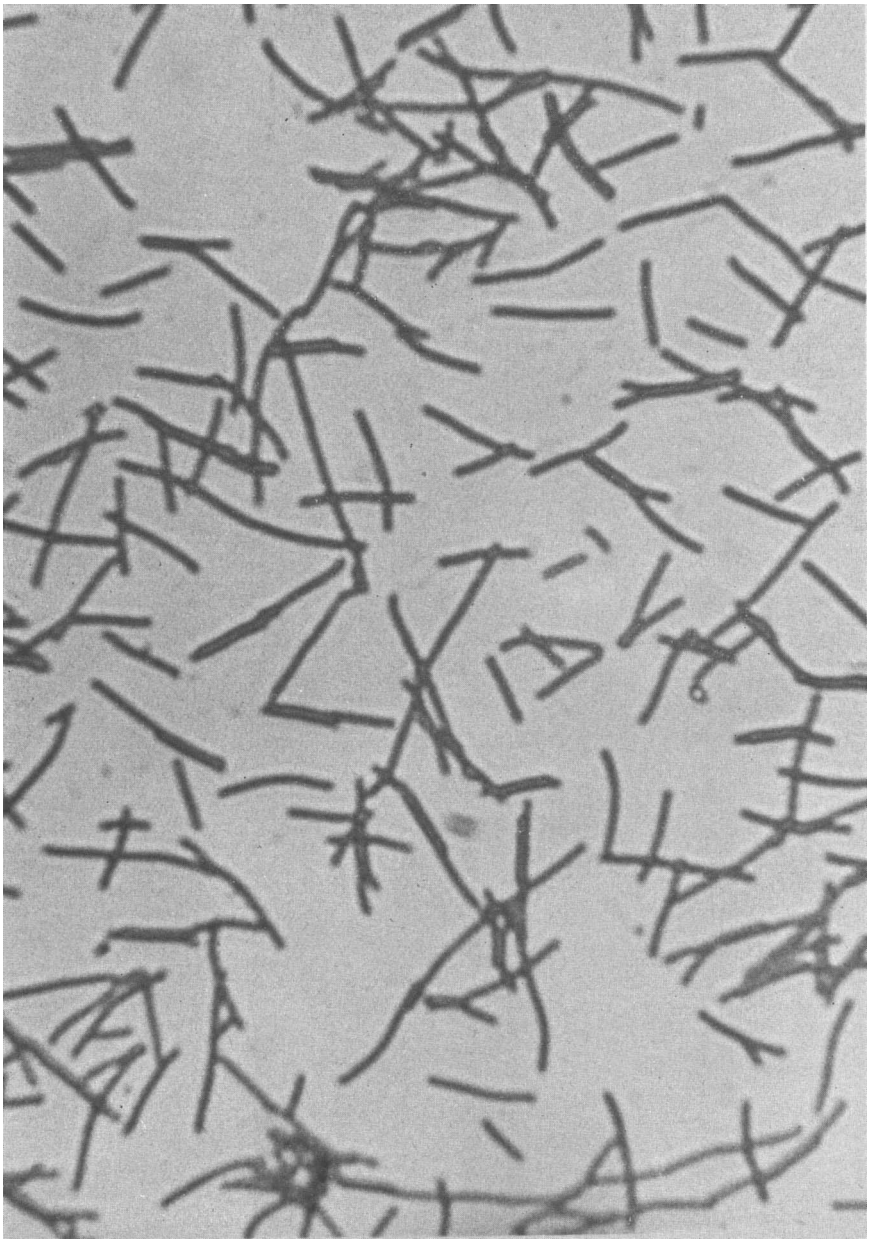

FIG. 2. Experimental *E. coli* culture, after logarithmic growth with 5-diazouracil for 1½ hr; cell count, $5 \times 10^8$ cells/ml; culture weight, 0.78 mg/ml (×475).

with *E. coli* have been described previously (1). Diazouracil at concentrations of 0.3 μg/ml completely inhibits division of *E. coli* without decreasing culture mass, producing morphologically uniform cultures of filaments which are 3 to 5 times the size of normal cells as shown in Fig. 1 and 2. In all assays controls consisted of cultures with and without diazouracil. In experiments to antagonize the division-inhibition, simultaneous additions of the inhibitor and the antagonizing agent were made. Growth measurements were based on culture dry weights; cell counts were made to determine the effect upon division. Two

methods of diazotization of primary aromatic amines were used; for amines which readily formed diazonium salts in aqueous solutions, the conventional method of diazotization was performed, *i.e.*, sodium nitrite was added to an equivalent quantity of the amine in an excess (3 eq) of mineral acid at 0-5°C(4). With difficultly-diazotizable amines concentrated nitric acid was added to the amine followed by the addition of an equivalent amount of a reducing agent, *e.g.* sodium metabisulfite(4). Spot tests by coupling the diazotization mixture with α-naphthol or α-naphthylamine were used to check qualitatively the effectiveness of diazotization(5).

*Results. Cell division inhibition studies.* The simplest aliphatic diazo compound, gaseous diazomethane, which was tested numerous times by various means, was shown, but only sporadically, to inhibit cell division of *E. coli*. Excessive amounts of diazomethane did inhibit growth, presumably by reacting with the glucose making it unavailable as a substrate for the culture, as shown by the failure of treated media to form a precipitate with 2,4-dinitrophenylhydrazine. The actual dose of diazomethane required to inhibit division could not be determined because of experimental difficulties in administering the gas to the cultures. However, the precursor of diazomethane, N-nitrosomethylurethane, at concentrations of 0.3 μg/ml promoted the formation of long filaments which were 3 to 5 times the size of the control cells. Other aliphatic diazo compounds, such as ethyl diazoacetate, diazoacetone, and diazoacetophenone, were either ineffective on division processes or at concentrations above 3 mg/ml were inhibitory. Diazotized aniline, 2-aminofluorene, and N,N-dimethyl-p-phenylene-diamine sulfate at concentrations of 30-10 μg/ml inhibit cell division of *E. coli*. p-Nitroaniline, 2,6-diaminopurine and p-aminobenzenesulfonamide when diazotized produced a slight inhibition of the organism. Certain other aromatic amines, pyrimidines, and purines were ineffective division inhibitors or were extremely toxic to growth.

*Antagonism of diazouracil inhibition.* Although the absence of an effect on the total culture mass with 0.3 μg diazouracil/ml seemed to preclude the concept that division inhibition resulted from a block in a major energy transfer system, adenosine triphosphate (ATP), adenylic acid, and diphosphopyridine nucleotide were tested for antagonistic activity. None of these compounds at concentrations of $5 \times 10^{-4}$ M or less alleviated the division block, although an exogenous source of ATP stimulated total culture growth by as much as 50%.

The additions of the intermediates of the glycolytic and the tricarboxylic acid cycles to diazouracil-inhibited cultures were without effect on the cell size of the bacteria. Yeast extract (Difco), Bacto-peptone, and casamino acids (hydrolyzed casein) were all observed to have slight antagonistic effects on inhibited cells and were observed to increase culture growth by 30 to 50%. These effects, however, were not striking and were obtained only at high concentrations (1-3 mg/ml).

L-Tyrosine at a concentration of 30 μg/ml completely antagonized the inhibition by diazouracil resulting in cells which were morphologically normal in appearance as shown in Fig. 3. 3,4-Dihydroxyphenylalanine, L-phenylalanine, L-serine, D- and L-tryptophans, tyramine, L-alanine, phenol, 3,5-diiodotyrosine, L-thyroxine, p-hydroxybenzoic, L-phenyllactic acid, p-hydroxyphenylglycine, and other amino acids were ineffective.

Such inhibition experiments, however, do not rule out the possibility that the antagonizing action of tyrosine to diazouracil is due merely to titrating out the inhibitor or to chemical inactivation of the inhibitor. Consequently, three additional types of experiments were performed: a) various mixtures of diazouracil and tyrosine were incubated at 37°C at pH 7 and pH 2. One-dimensional chromatograms in butanol-acetic acid-water solvent of these mixtures were compared with chromatograms of tyrosine. In all cases the $R_f$ values and the intensity of the ninhydrin developed spots of the mixtures were identical with those of appropriate tyrosine controls; b) the ultraviolet absorption spectra of incubated mixtures of tyrosine and diazouracil showed no shifts in absorption maxima from the spectra of tyrosine and diazouracil alone; c) the spectrophotometric de-

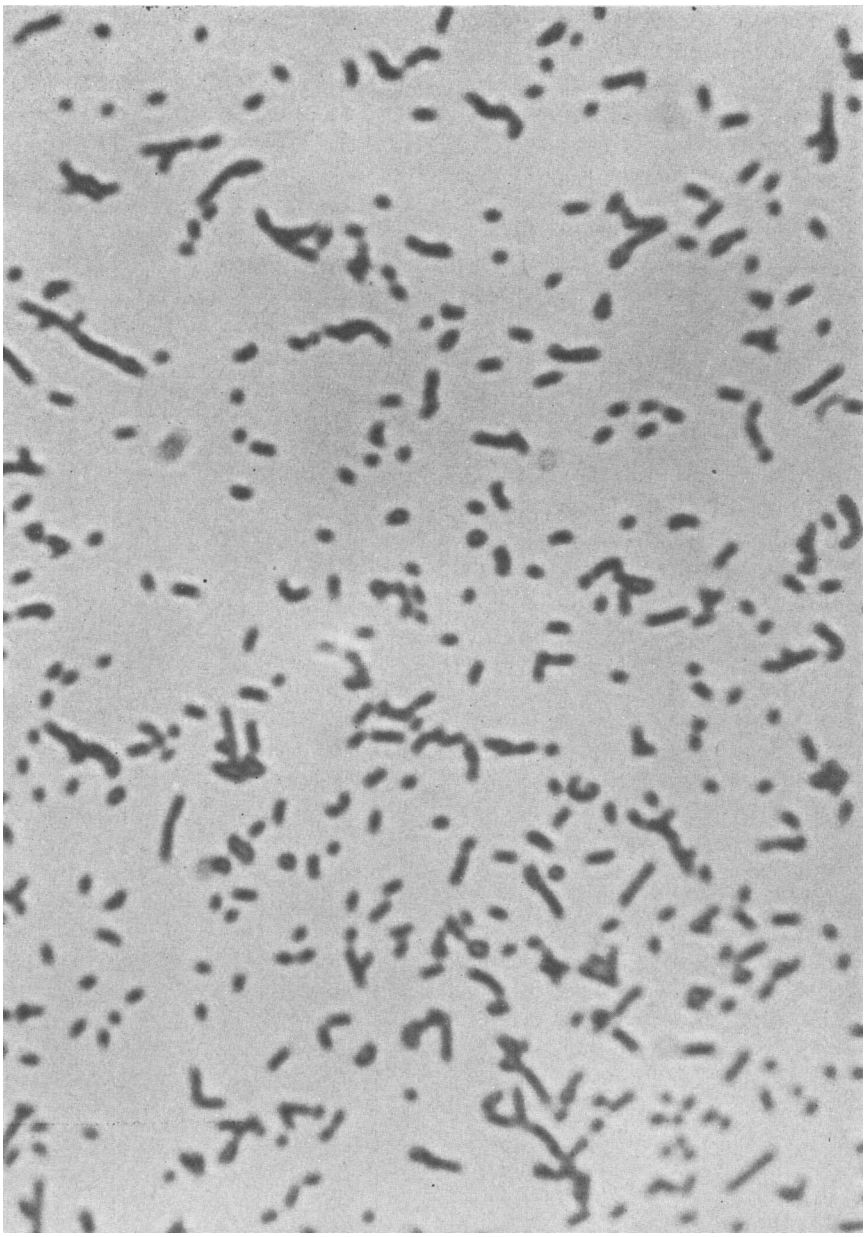

FIG. 3. Experimental *E. coli* culture treated with 5-diazouracil and with tyrosine; cell count, 1.5 $\times$ $10^9$ cells/ml; culture weight, 0.78 mg/ml ($\times$475).

termination at 540 m$\mu$ of diazouracil and incubated diazouracil-tyrosine mixtures after coupling with $\alpha$-naphthylamine showed no decrease in the diazouracil concentration. The evidence from these experiments indicates that there is no interaction between diazouracil and tyrosine; consequently, the antagonism of diazouracil inhibition by tyrosine is not a result of the destruction of diazouracil.

In contrast to these observations it can be shown that sodium hydrosulfite and ascorbic acid, but not dehydroascorbic acid, antagonize diazouracil inhibition by inactivating the diazouracil. Diazouracil activity can also be destroyed by pretreatment with alkali.

*Discussion.* While all the synthesized diazo

compounds were not effective cell-division inhibitors, the structures of the effective compounds are so dissimilar that the activity of these compounds as inhibitors appears to be due to a functional group specificity rather than a total structural specificity. Such an hypothesis would indicate that the activity of diazouracil is due to the diazo portion of the molecule rather than to the uracil moiety. Additional work is being conducted in an attempt to determine whether diazo groups at positions other than the 5-position of the pyrimidine molecule are as effective in inhibiting cell division.

Investigation of the relationship between cell-division inhibition by diazouracil and tyrosine antagonism is being continued in an attempt to determine the role of tyrosine in cell division. It is apparent that inhibition of cell division in these experiments was not a result of a block in reactions dependent on high-energy phosphate. Other work in this laboratory has shown that there are no significant changes in the phosphorus fractions of these inhibited *E. coli* systems(3). It may be that tyrosine and/or aromatic amino acid metabolism are involved in the inhibition of cell division.

*Summary.* Studies were made to determine the specificity of the chemical inhibition of division of *Escherichia coli* and to determine the relationship of division inhibition to important biochemical metabolites. It was found that the cell-division process of *E. coli* could be specifically inhibited, without an accompanying inhibition of culture growth, by diazouracil and by other diazonium compounds, and it is suggested that the diazo group on the compounds is responsible for the inhibition. The inhibition of division by diazouracil was antagonized by tyrosine, and it was shown that the antagonism can not be attributed to a destruction of the inhibitor by tyrosine. Other amino acids, intermediate in the glycolytic and tricarboxylic acid cycles, and energy-rich phosphorus compounds were incapable of antagonizing diazouracil-inhibited cultures.

1. Loveless, L. E., Spoerl, E. S., and Weisman, T. H., submitted to *J. Bact.*

2. Spoerl, E. S., Loveless, L. E., Weisman, T. H., and Balske, R. J., *J. Bact.*, 1954, v67, 394.

3. Katchman, B. J., personal communication.

4. Karrer, P., *Organic Chemistry,* 2nd English Edition, Elsevier Publishing Co., New York, N. Y., 1946, pp 455-6.

5. Feigl, F., *Qualitative Analysis by Spot Tests,* Nordeman Publishing Co., Inc., New York, N. Y., 1939, p322.

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## Role of Antral Exclusion in Development of Peptic Stomal Ulcer.* (21072)

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The importance of the chemical phase of gastric secretion is well known. This phase is mediated through the production of the hormone gastrin in the antrum of the stomach when food or its digestion products come in contact with the antrum. Dragstedt *et al.* (1,2) have shown that transplantation of the antrum to the colon produces a profound and sustained increase in the secretion of hydrochloric acid from an isolated gastric pouch and also that it causes the development of peptic stomal ulcers in a high proportion of dogs. More than 30 years ago Koennecke(3) had shown that placement of the antrum upon the distal ileum of dogs invited stomal peptic ulcers. Recently, Sauvage *et al.*(4) reported a series of experiments which suggested that the antrum was the most important single factor in the control of gastric secretion. However, all of the experiments reported by Sauv-

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