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Potentiated Inhibition of *Escherichia coli* by Certain Combinations of Agents. (22490)

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Much useful information has been obtained on the modes of action of chemotherapeutic agents, including tumor-inhibiting agents, by inhibition analysis in bacterial systems. In a number of cases the mode of action appears to be the same in bacterial and mammalian systems. Therefore, it is not unreasonable to test agents or combinations of agents against bacteria to serve as a guide for tests in mammals. In this laboratory, candidate anticancer agents are tested singly and in combinations as growth inhibitors for bacteria prior to testing against neoplasms in animals. This report presents some of the data that have been obtained for combinations of agents in tests with bacteria.

Methods. To screen for potentiating growth-inhibitory activity, a simple test method was devised using *Escherichia coli* (ATCC No. 9637) as the test organism. The use of this organism grown in a simple, chemically defined glucose-salts medium is ideal for biochemical studies of this kind because the observed action of specific inhibitors or antimetabolites is not obscured by the presence in the medium of the chemically complex metabolites required in similar studies on more fastidious organisms. Duplicate paper discs saturated with known concentrations of a candidate compound were placed on the surfaces of minimal agar cultures of *E. coli* in a control plate and in a test plate. The control plate contained no test compound, whereas the test plate contained a subinhibitory concentration of the second member of the pair of candidate compounds being tested for potentiation. If the diameter of the zone of inhibition around the paper disc on the test plate was greater than 2 times that on the control plate, then the combination was considered worthy of further study. Of approximately one thousand pairs of compounds tested, approximately one hundred pairs gave evidence of significant additive or potentiated inhibition. Although the agar-plate method described above was quite useful for rapidly screening combinations for additive activities, it was considered desirable to subject the combinations that showed the more promising activities to another test, which would permit a quantitative evaluation of the combinations. For this purpose, the method of Elion, Singer, and Hitchings(1) was used, and *E. coli* (ATCC No. 9637) was used instead of *Lactobacillus casei* or *Streptococcus faecalis*. The minimal medium of Davis and Mingioli(2) was used with the omission of the agar. A 24-hour culture of *E. coli* was adjusted by addition of sterile water to have a transmission of 55 $\pm$ 2% (Bausch and Lomb Monochromatic Colorimeter with a 660 mμ interference filter), and then diluted 1:10 with sterile water. Each 10-ml tube of medium was inoculated with 0.1 ml of this suspension. The extent of growth was determined turbidimetrically after incubation at 37° for 16-18 hours. The effects of the 2 compounds being tested upon the growth of the bacteria were determined by testing various levels of the compounds alone and in combination. In each case, the quantity of the compound required to cause half-maximal inhibition was determined graphically as shown in Fig. 1 and 2. The fractional inhibitory concentra-

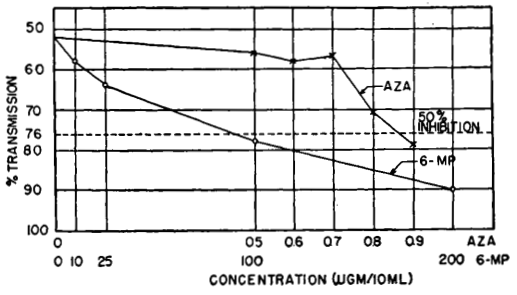

FIG. 1. Inhibition of growth of *E. coli* by azaserine and by 6-mercaptopurine.

TABLE I. Concentrations of Azaserine and 6-Mercaptopurine Causing Half-Maximal Inhibition of Growth of *Escherichia coli.*

Azaserine		6-Mercaptopurine	
μg/10 ml	Fractional inhibitory conc.	μg/10 ml	Fractional inhibitory conc.
.00	.00	88.8	1.00
.10	.12	8.7	.10
.15	.18	7.3	.08
.20	.23	4.4	.05
.25	.29	3.4	.04
.30	.35	3.0	.03
.86	1.00	0.0	.00

tion for each compound in a particular combination was calculated by dividing the concentration of the inhibitor present in the combination by the quantity of inhibitor which would be required to give half-maximal inhibition by itself. (Table I). By plotting the fractional inhibitory concentrations as shown in Fig. 3, it is possible to determine the effectiveness of the combination. If the 2 compounds have effects which are additive, the points will fall on a straight line connecting unity on the ordinate with unity on the abscissa. Deviations to the left of this theoretical straight line indicate potentiation; deviations to the right might represent interference or antagonism between the drugs. By drawing intersecting straight lines through the experimental points, a point is obtained where the combined fractional inhibitory concentrations reach a minimum. This point represents the point of maximal effectiveness. The numerical sum of the coordinates of this point can be used to indicate the degree of effectiveness of the combination—the smaller the value of the sum, the more effective the combination. Therefore, by comparing the magnitudes of these sums, it is possible to compare the activities of various combinations. Sums that were determined from data obtained in these experiments are presented in Table II. Each experiment was performed in duplicate or triplicate, and each sum listed in Table II is the mean value for these experiments. To permit correlation of antibacterial activity and anticancer activity, the results of some tests with Sarcoma 180, Adenocarcinoma 755, and Leukemia L1210 are indicated in Table II. Some of the combinations are listed twice in the table to facilitate comparison with the values for other combinations.

Comments. The usefulness and limitations of this type of experiment have been discussed (1), and the chief purpose of the present paper is to present the data for combinations that have been tested in this laboratory. Although these data could be used in discussions of biochemical mechanisms, such discussions will not be presented here.

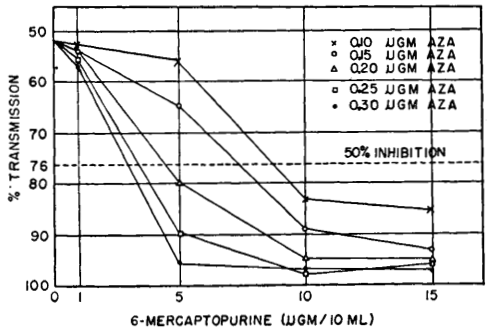

FIG. 2. Inhibition of *E. coli* by combinations of azaserine and 6-mercaptopurine.

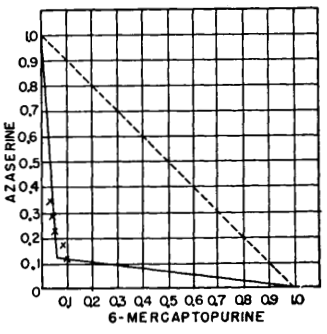

FIG. 3. Effects of combinations of azaserine and 6-mercaptopurine upon growth of *E. coli* in terms of fractional inhibitory concentrations.

Several combinations were found to be outstandingly potentiating. Perhaps the combinations of Daraprim with canavanine sulfate and of 3-(1,2,4-triazolyl)alanine with canavanine sulfate were the most active, since the values presented in Table II for these combinations were based upon subinhibitory levels of canavanine sulfate and the true values would therefore be lower than the tabulated values.

TABLE II. Effectiveness of Combinations of Drugs.

Combination	Sum of fractional inhibitory conc. of most effective combination for *E. coli*	Potentiation of anticancer activity* Positive	Negative
Sulfanilamide + Desoxypyridoxine	.24		L1210†
+ Adenine sulfate	.39	Not tested	
+ Daraprim‡	.55		L1210† Sa 180†
+ 6-Mercaptopurine	.57	L1210† Sa 180† (?)	
+ Hypoxanthine§	.58		L1210† Sa 180†
+ DL-Ethionine	.59	L1210†	Sa 180†
+ 3-(1,2,4-Triazolyl)alanine	.67		L1210†
Azaserine‖ + 6-Mercaptopurine	.30	L1210 Sa 180	
+ 3-(1,2,4-Triazolyl)alanine	.47		L1210
+ 2,6-Diaminopurine	.67	Not tested	
+ Desoxypyridoxine	.76	Ad 755 Sa 180	L1210
Daraprim + Canavanine sulfate§	.13		L1210
+ 2,6-Diaminopurine	.47	Not tested	
+ Sulfanilamide	.55		L1210 Sa 180
Desoxypyridoxine + 3-(1,2,4-Triazolyl)alanine	.13		Sa 180
+ DL-Ethionine	.23		Sa 180
+ Sulfanilamide	.24		L1210
+ Benzoic acid hydrazide	.32	Sa 180	
+ Isonicotinic acid hydrazide	.43	"	
+ p-Aminobenzoic acid hydrazide	.52	"	
+ 1, 5-Diaminobiuret	.67	"	
+ Azaserine	.76	Ad 755 Sa 180	L1210
+ Methionine sulfoximine	1.21	Not tested	
3-(1,2,4-Triazolyl)alanine + 6-Mercaptopurine	.09		L1210
+ Canavanine sulfate§	.11		L1210
+ Desoxypyridoxine	.13		Sa 180
+ Adenine sulfate	.14	Not tested	
+ 6-Chloropurine	.15	"	
+ 2, 6-Diaminopurine	.15	"	
+ 5-Bromouracil	.44	"	
+ Azaserine	.47		L1210
+ 2-Thiazolealanine	.60	Not tested	
+ Sulfanilamide	.67	"	
Streptomycin sulfate + 6-Mercaptopurine	.63	"	
+ Dithiouracil	.79	"	
+ Azaserine	.84	"	

* The experimental neoplasms used were Leukemia L1210, Sarcoma 180, and Adenocarcinoma 755.

† In tests of anticancer activity, A-methopterin was used instead of sulfanilamide.

‡ 2, 4-Diamino-5-p-chlorophenyl-6-ethylpyrimidine.

§ Calculations were based upon a subinhibitory level of this compound, and therefore, the combination is perhaps much more active than figure in table indicates.

‖ O-Diazoacetyl-L-serine.

It is interesting to note that the combinations that were most effective against *E. coli* were often ineffective in the tests against neoplasms, whereas some combinations that were less effective against *E. coli* were effective against these neoplasms. For example, although the value of the sum of the fractional inhibitory concentrations for azaserine plus 6-mercaptopurine is 0.30 and that for 3-(1,2,4-triazolyl)alanine plus 6-mercaptopurine is 0.09, the former combination was effective against leukemia L1210 whereas the latter was ineffective. Other similar examples could be picked from the table. On the other hand, combinations that have been found to be potentiating in tests with animal neoplasms are also potentiating in inhibiting the growth of *E. coli*. Although the correlation between the data for *E. coli* and the indicated neoplasms is not as good as would be desired, the bacterial screen does indicate combinations that should be tested against neoplasms in animals. For example, the potentiating effects of combinations of desoxypyridoxine and acid hydrazides were first detected in tests with bacteria.

It is also possible that some of the combinations that are potentiating against *E. coli* might be of value in studies related to the chemotherapy of infectious diseases caused by bacteria or protozoa.

Summary. Data are presented for the potentiating effect of several combinations of compounds in inhibiting the growth of *E. coli*. Data of this kind can serve as guides for the selection of combinations of agents to be tested in the chemotherapy of cancer and of infectious diseases.

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Tremor Production in Cats Given Chlorpromazine. (22491)

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The production of a Parkinson-like state in humans receiving large daily doses of chlorpromazine has been observed clinically by us and others(1-4). To study further this phenomenon, chlorpromazine in different dosages and for variable periods of time, was injected in cats and the results observed. It was anticipated that a state of rigidity would be most prominent if any effects were obtained, but an unexpected feature was the appearance of an alternating tremor at rest. This has previously been produced in primates by fulgurations in the region of the subthalamus(5,6), ablations of the cerebellar cortex and nuclei (7), fulgurations in the mesencephalic and pontine tegmentum(8), electrical stimulation of the medial reticular formation(9), and by use of Reserpine(10). Folkerts and Spiegel (11) observed an alternating tremor during electrical stimulation of the midbrain tegmentum in the cat.

Methods. Twenty-eight adult cats were observed prior to and following the intramuscular injection of chlorpromazine to determine the quality of placing and hopping reactions, labyrinthine righting reactions, stütz response, both dependent and supine, pupillary reaction, general performance, gait, tendon reflex activity, muscle tonus, and tremor. Drug doses were calculated on the basis of those frequently employed for a 70 kg human which range from 300 to 2500 mg/day and were proportioned according to the cat's weight. Doses were given once daily. They varied in amount as mentioned and were administered as single injections, intermittent injections, or consecutive daily injections for as long as 30 days. Tremors and other drug