irradiation (X-ray) on Ca755, KB, and HeLa (S-3) cells were potentiated by hadacidin. Under similar experimental conditions, no potentiation of the lethal effect was seen with Sa180 cells and the results with H.Ep-2 cells were inconsistent and equivocal. With Ca755, KB, and HeLa (S-3) cells, the degree of potentiation was related to the dose of both X-ray and hadacidin.

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Hadacidin Potentiation of Lethal Action of Ionizing Irradiation. II. Effect on Proliferating Gram-Negative Bacteria.* (29896)

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The antibiotic, N-formyl hydroxyaminoacetate, produced by *Penicillium frequentans* Westling and trivially named hadacidin(1), has been reported to be an inhibitor of tumor growth(2,3). Shigeura and Gordon have shown that one site of action of hadacidin is the inhibition of the conversion of inosinic acid to adenylosuccinic acid by adenylsuccinate synthetase(4). Recently, it has been reported that hadacidin potentiates the lethal action of ionizing irradiation on Eagle's KB cells(5). The activity of hadacidin as a radiopotentiator in microbial systems has been investigated.

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Methods. The following procedures were used for determining the effect of hadacidin on the action of ionizing irradiation on growing bacterial cells: The bacteria used were grown for 18 hours in a simple, synthetic, glucose-salts medium (NH_4Cl, 1.0 g; K_2HPO_4, 7.32 g; KH_2PO_4, 3.0 g; $MgSO_4$, 0.12 g; deionized water, 1 liter. After autoclaving at 15-lb pressure for 30 minutes, 8 ml of sterile 50% glucose was added). The cultures were thrice washed in M/15 phosphate buffer (pH 7.0) and standardized to 20% light transmittance on a Bausch and Lomb "Spectronic 20" spectrophotometer (660 mμ) with the buffer. Replicate tubes of synthetic medium, with and without hadacidin in varying concentrations, were inoculated with 0.1

TABLE I. Hadacidin Potentiation of Ionizing Irradiation (Co^{60}) in *Escherichia coli* ATCC 9637.

Growth time (hr)		Unirradiated controls	% Survivors irradiated—20 Kr
		Without hadacidin	
0	(a)	1.0×10^{7}*	.3
2	(b)	2.0×10^{7}	.5
4	(c)	1.0×10^{8}	.2
6	(d)	7.0×10^{8}	.7
		With 0.01M hadacidin	
		%†	
0	(a′)	100	.009
2	(b′)	50	<.0001
4	(c′)	40	<.00003
6	(d′)	10	.001
		With 0.001M hadacidin	
		%†	
0	(a″)	100	.06
2	(b″)	50	.008
4	(c″)	80	<.00001
6	(d″)	71	.001
		With 0.0001M hadacidin	
		%†	
0	(a‴)	100	.02
2	(b‴)	100	.001
4	(c‴)	200	<.00005
6	(d‴)	100	.09

* Numbers refer to viable organisms/ml as determined by plate count.

† Percent survivors after exposure to compound in absence of irradiation (a′/a, a″/a, a‴/a, b′/b . . .).

ml of the cell suspensions (final volume was 10 ml) and incubated at 37°C. According to the needs of the experiment, representative tubes were removed, placed in an ice bath, and tube contents divided into aliquots some of which were irradiated, the rest served as unirradiated controls. Irradiation was accomplished with the use of a Co^{60} source (ca. 500 curies) at a rate of 2.5 Kr/minute under our conditions of exposure. After irradiation all samples, both irradiated and unirradiated controls, were diluted in 0.85% saline and plated in triplicate in Trypticase Soy Agar, Baltimore Biological Laboratories (BBL). The plates were incubated 18-24 hours at 37°C, visible colonies counted, and numbers of viable cells/ml original broth determined.

Results and discussion. Typical data illustrating the effectiveness of hadacidin as a radiopotentiator for *Escherichia coli* ATCC 9637 are presented in Table I. It may be noted that concentrations of hadacidin which

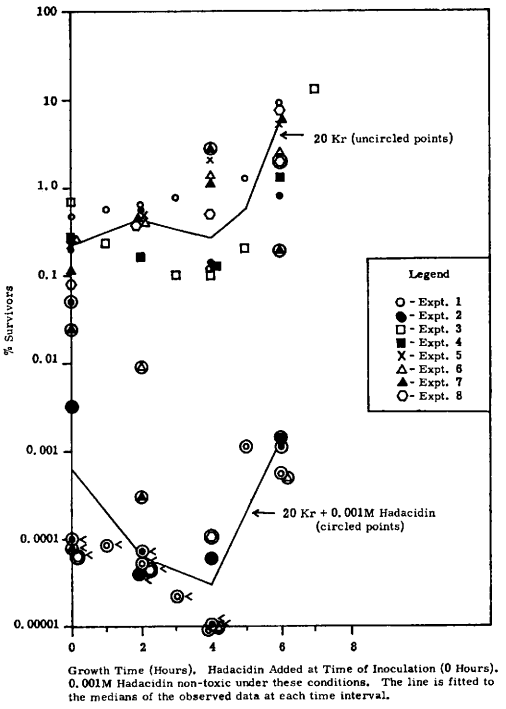

FIG. 1. Potentiation of lethal effect of ionizing irradiation on growing *Escherichia coli* ATCC 9637 by hadacidin.

alone are nontoxic for *E. coli* cause significantly more kill in combination with irradiation than does irradiation alone. For example, exposure of *E. coli* cells that have been growing for 4 hours to 20 Kr results in death of 99.9% of the cells. Exposure of similar cells grown for 4 hours in the presence of a nontoxic concentration of hadacidin (0.001 M) and exposed to 20 Kr results in the death of more than 99.9999% of the cells.

Considerable variation in sensitivity of proliferating *E. coli* ATCC 9637 to both ionizing irradiation and degree of potentiation caused by hadacidin has been noted. Fig. 1 presents graphically the results of 8 pooled experiments with *E. coli* ATCC 9637, hadacidin, and ionizing irradiation. It is obvious that hadacidin does increase the effects of ionizing irradiation on *E. coli* ATCC 9637 regardless of experiment-to-experiment variation.

The radiation potentiating properties of hadacidin have been demonstrated in a variety of other Gram-negative bacteria. Table II illustrates hadacidin potentiation of ioniz-

ing irradiation kill of *E. coli* B (ORNL) and the derived irradiation-resistant strain designated *E. coli* B/r(6), the Hill culture of *E. coli* B and the derived irradiation-sensitive strain designated *E. coli* B_s(7), *E. coli* ATCC 11303, *Salmonella typhimurium* SR-11 and *Serratia marcescens* ATCC 274. Two alkylating agent-resistant strains of *E. coli* [one derived from *E. coli* ATCC 9637 as resistant to nitrogen mustard and designated *E. coli*/HN2(8), the other derived from *E. coli* B (Hill) as resistant to 1,3-bis(2-chlorethyl)-1-

TABLE II. Potentiation by Hadacidin of Ionizing Irradiation Kill in a Number of Bacterial Strains.

Bacterium	Time (hr) following addition of hadacidin	Without hadacidin		.001M hadacidin	
		Bacteria/ml unirradiated controls	% Survivors irradiated —20 Kr	% Survivors no irradiation	% Survivors irradiated —20 Kr
Escherichia coli ATCC 9637	0	1.0×10^7*	.3	100	<.0001
	2	2.0×10^7	.4	100	.01
	4	1.0×10^8	2.0	25	<.00001
	6	6.0×10^8	3.3	83	.0006
Escherichia coli ATCC 9637/HN2†	0	9.0×10^6	3.3	100	.0008
	2	2.0×10^7	2.5	100	.0002
	4	2.0×10^8	1.0	100	.00003
	6	6.0×10^8	8.3	50	.01
Escherichia coli ATCC 9637/FRM†	0	1.0×10^7	.1	100	.002
	2	3.0×10^7	.02	100	.001
	4	2.0×10^8	.3	50	.4
	6	1.0×10^9	8.0	50	.4
Escherichia coli B (ORNL)	0	2.0×10^7	.005	100	<.0001
	2	4.0×10^7	.002	100	<.0025
	4	3.0×10^8	.001	67	<.00005
	6	1.0×10^9	.2	67	.25
Escherichia coli B/r†	0	1.0×10^7	20.0	200	10.0
	2	2.0×10^7	5.0	100	.01
	4	1.0×10^8	3.0	100	.002
	6	7.0×10^8	14.0	71	4.0
Escherichia coli B (Hill)	0	1.0×10^7	.1	100	.8
	2	2.0×10^7	.05	100	.005
	4	1.0×10^8	.03	100	.00001
	6	5.0×10^8	.02	8	.001
Escherichia coli B (Hill)/BCNU†	0	2.0×10^7	3.0	100	1.0
	2	3.0×10^7	.3	100	.001
	4	2.0×10^8	—	100	—
	6	7.0×10^8	4.3	71	.2
Escherichia coli B_s (Hill)	0	2.0×10^7	.03	100	.0003
	2	3.0×10^7	.02	100	.00003
	4	2.0×10^8	.01	50	.00007
	6	9.0×10^8	.1	67	.008
Escherichia coli ATCC 11303	0	1.0×10^7	.009	100	.0002
	2	2.0×10^7	.01	100	.001
	4	1.0×10^8	.001	90	.0002
	6	6.0×10^8	.3	83	.002
Salmonella typhimurium SR-11	0	9.0×10^6	.2	111	.0001
	2	3.0×10^7	.3	67	.00005
	4	1.0×10^8	.5	70	.3
	6	4.0×10^8	2.5	75	.01
Serratia marcescens ATCC 274	0	2.0×10^7	.002	100	.0003
	2	4.0×10^7	.01	100	.0008
	4	1.0×10^8	.02	90	.0001
	6	4.0×10^8	.05	50	.0001

* Numbers in table refer to viable cell counts/ml as determined by colony counts in Trypicase Soy Agar.

† / Denotes resistance thus: Name of parent culture/compound to which strain is resistant.

HN2 = nitrogen mustard; FRM = n-methylformamide; r = irradiation; BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea.

TABLE III. Hadacidin's Radiopotentiating Ability as a Function of Physiological Age of *Escherichia coli* Cells.

Time (hr) following inoculation when hadacidin added	Hours growth	Without hadacidin		.001M hadacidin	
		Unirradiated controls	% survivors irradiated–20 Kr	% survivors no irradiation	% survivors irradiated–20 Kr
0	0	9.0×10^{6}*	.4	111	<.0001
	1	1.0×10^{7}	.8	100	<.0001
	2	2.0×10^{7}	.5	100	<.00005
	3	6.0×10^{7}	.7	67	<.00003
	4	2.0×10^{8}	.1	100	<.00001
	5	4.0×10^{8}	1.5	50	.002
	6	9.0×10^{8}	10.0	67	.0007
2	2	2.0×10^{7}	.3	100	<.00005
	3	7.0×10^{7}	.06	71	<.00002
	4	2.0×10^{8}	.005	100	.00004
	5	6.0×10^{8}	6.7	50	.0001
	6	9.0×10^{8}	7.8	67	.3
4	4	2.0×10^{8}	.05	50	<.00001
	5	3.0×10^{8}	.7	133	.05
	6	6.0×10^{8}	3.3	17	—
6	6	6.0×10^{8}	1.3	67	.8

* Numbers refer to viable cells/ml as determined by plate counts.

nitrosourea and designated *E. coli*/BCNU (9)] were both sensitive to the radiopotentiating action of hadacidin. Similarly a strain of *E. coli* resistant to N-methylformamide [derived from *E. coli* ATCC 9637 and designated *E. coli*/FRM(8)] was also sensitive to the radiopotentiating action of hadacidin.

Plating Co^{60}-irradiated *E. coli* ATCC 9637 cells in agar medium containing hadacidin at concentrations as high as 0.01 M resulted in the same percentage of survivors as were obtained when the irradiated cells were plated in hadacidin-free medium. It may be concluded that hadacidin must be present prior to or during irradiation in order to exert its effect.

In preliminary studies with *E. coli* ATCC 9637 it has been observed that the dosage of irradiation must be high enough to be in itself significantly lethal to the cells before hadacidin is effective as a radiation potentiator.

The effect of the amount of time growing cells are exposed to hadacidin has on the compound's irradiation-potentiating ability has been investigated. Also, the sensitivity of cells from different points in the growth cycle to the hadacidin effect has been determined. Several tubes of the mineral salts medium described above were inoculated with *E. coli* ATCC 9637 and incubated at 37°C for 6 hours. At zero time (inoculation time) hadacidin at concentrations of 0.01 M, 0.001 M, and 0.0001 M was added to several of the cultures. After 2 hours incubation, hadacidin was added to a second set; after 4 hours, hadacidin was added to a third set; and after 6 hours, hadacidin was added to a fourth set of cultures. At hourly intervals post-addition of hadacidin, samples were taken from each set, placed in ice, irradiated with 20 Kr from Co^{60}, and plated in triplicate in Trypticase Soy Agar along with unirradiated controls. The plates were counted after overnight incubation. The results with 0.001 M hadacidin presented in Table III suggest that, regardless of how long *E. coli* ATCC 9637 cells are exposed to hadacidin or at what time the compound is added, hadacidin potentiates the lethal effects of ionizing irradiation.

Summary. It may be concluded from this work that hadacidin is a potent potentiator of the lethal effects of ionizing irradiation on proliferating cells of a number of Gram-negative organisms. It appears from preliminary studies that for hadacidin potentiation of ionizing irradiation to be demonstrated, a damaging dose of irradiation must be used and that the compound must be present either prior to or during irradiation. Our data also suggest that hadacidin potentiates irradiation damage regardless of the physiological age of

the cells at the time of addition of hadacidin or the amount of time the cells are exposed to it during growth.

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Mental Functioning and Premarin Therapy in Cardiovascular and Cerebrovascular Disease.* (29897)

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Improved mental functioning has been reported in patients with atherosclerosis treated with conjugated equine estrogens (Premarin) in comparison with similar patients given placebos(1). Improvement was evident in patients with myocardial infarction and those with cerebral thrombosis. Caldwell and Watson(2,3) also reported improved intellectual functioning in aged women treated with estrogens, but Lifshitz and Kline(4) found no difference between estrogen- and placebo-treated men with psychosis associated with cerebral atherosclerosis. In studying sex hormone replacement in the elderly, Pauker, Kheim, Mensh, and Kountz(5) observed no difference in post-therapy psychological evaluations between patients treated with androgens-estrogens and control patients.

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The purpose here is (a) to report a second testing of the hypothesis that mental impairment decreases in patients with cardiovascular and cerebrovascular disease treated with Premarin, and (b) to present an analysis of the results with patients from the two studies combined. The hypothesis was tested with the use of the Rorschach inkblot technique, a sensitive indicator of mental impairment accompanying organic damage to the brain.

Methods. Included in the study were 77 new male patients who were participating in a prolonged clinical trial of the effects of Premarin therapy in cardio- and cerebrovascular disease at the Los Angeles County Hospital. There were 45 men who had recovered from an acute myocardial infarction and 32 who had had a cerebral thrombosis. After careful medical evaluation to ensure accurate diagnosis, the patients were randomly assigned to Premarin or placebo treatment by a statistical control office, and were thereafter seen in special outpatient clinics at approximately 6-week intervals. Relatively small