of UDPGA, this reaction did not occur if the soluble fraction of the cell was also present. This confirms the results of earlier experiments(4,5) with rat liver slices. Yet, under identical conditions, marked glucosiduronate formation took place with the 8000 $\times$ *g* supernatant (microsomes + soluble fraction) of guinea pig liver, even without addition of UDPGA. Furthermore, it was shown by chromatography and autoradiography that a compound with the R_f of estradiol-17β-glucosiduronate was present in those guinea pig and rat liver fractions where conjugation was indicated (Table II) and that this radioactive product disappeared after hydrolysis with β-glucuronidase.

Thus, although rat liver microsomes are able to conjugate estrogens with glucuronic acid, no such reaction occurs when the soluble fraction of the cell is also present and a different type of compound must be postulated to account for the water-soluble ^{14}C-metabolites that appear under these conditions. They could be very polar polyhydroxylated estrogen derivatives, degradation products in which ring opening has occurred or else unhydrolyzed conjugates of unknown nature. This species difference must be exerted at the microsomal level because the same products were formed by rat liver microsomes irrespective of whether the soluble fraction was derived from rats or guinea pigs, and there is some evidence(7) that glutathione may be involved.

Work is in progress to identify these unknown metabolites and to determine whether any other species metabolize estrogens in this atypical manner since it may have some bearing on the ease with which mammary tumors can be induced in the rat(8) by estrone pellets.

Summary. A marked species difference in estrogen metabolism has been observed between the rat and the guinea pig, both *in vivo* and in incubations with subcellular fractions of liver. Very little, if any, estrone-16-^{14}C administered to rats appeared in the urine as the sulfate or glucosiduronate and although the liver microsomes from this species were shown to be able to conjugate estrogens with glucuronic acid, no such reaction occurred if the soluble fraction of the cell was also present. In contrast, under identical conditions, large amounts of the normal estrogen conjugates were formed by the guinea pig.

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Received October 26, 1964. P.S.E.B.M., 1965, v118.

Hadacidin Potentiation* of Lethal Action of Ionizing Irradiation. I. Effect of Mammalian Tumor Cells in Culture.† (29895)

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The antibiotic hadacidin (N-formylhydroxyamino acetic acid), isolated from culture filtrates of *Penicillium frequentans* Westling(1) has been reported to be an inhibitor of tumor growth(2,3) and to potentiate the cytotoxicity of phenethylbiguanide for KB cells in culture(4). Since phenethylbiguanide has been reported to stimulate glycolysis in mammalian cells(5) and the radiation response of mammalian and other cells varies with the oxidation state(6) it was of interest to ex-

* Potentiation — a degree of synergism that is greater than additive.

† The work reported here was supported by the Cancer Chemotherapy National Service Center, Nat. Cancer Inst., under NIH Contract PH-43-62-485.

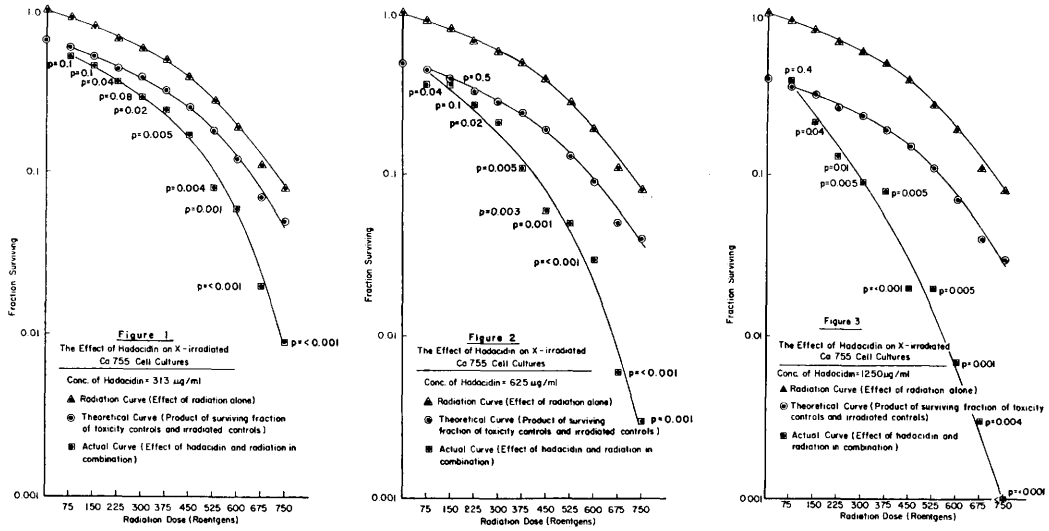

FIG. 1-3

amine the effect of hadacidin on radiation damage to mammalian cells.

Materials and methods. KB(7), H.Ep-2 (8), HeLa (S-3)(9), Ca755,‡ and Sa180(10) cells were grown in stationary cultures in Eagle's basal medium(11) supplemented with 10% calf serum. Cells in the log phase of growth were harvested by trypsinization. Plastic cell culture bottles§ were seeded with varying sized cell inocula in SRI No. 14 medium(12) supplemented with 10% calf serum and containing selected concentrations of hadacidin. The number of cells seeded into each bottle was adjusted so that irradiated control cultures counted 11 to 14 days after irradiation contained 30 to 300 clones. After incubation for 24 hours at 37°C, the cultures were exposed to varying doses of radiation from a 140-kV, 8-mA, X-ray source, adjusted to deliver 75 r per minute. Uniform radiation doses were obtained by placing the cultures on a turntable rotating at 13 rpm. Each radiation exposure was monitored with an ionization chamber. Immediately following irradiation, the medium from all cultures (including nonirradiated, non-hadacidin-treated controls; nonirradiated, hadacidin-treated controls; irradiated, non-hadacidin-treated controls; and irradiated, hadacidin-treated test cultures) was removed and replaced with fresh medium without hadacidin. The cultures were incubated at 37°C and refed on the seventh day. On the eleventh day, clones suitable for counting were stained with Giemsa stain and counted; if they were too small for accurate counting on the eleventh day, the cultures were refed and the clones were stained and counted on the fourteenth day.

In evaluating the experimental data, the fraction of surviving cells treated with hadacidin alone was determined and the fraction of surviving cells treated with hadacidin plus radiation was corrected for hadacidin toxicity (see Fig. 1-3 for hadacidin toxicity to Ca755 cells). The theoretical fraction of surviving cells treated with hadacidin plus radiation was assumed to be the product of the fraction of cells surviving hadacidin treatment alone and the fraction surviving radiation treatment alone (assumption of additive cytotoxicity). It was further assumed that the dose of hadacidin used to determine the fraction of surviving cells in hadacidin toxicity control cultures would not be more toxic to the larger cell populations used in the cultures exposed to hadacidin plus radiation. It was necessary to expose larger numbers of cells to hadacidin plus radiation to assure cultures containing between 30 and 300 clones.

‡ Mouse mammary adenocarcinoma 755, established in cell culture at Southern Research Institute.

§ 30-ml capacity sealed bottles (Falcon Plastics, Los Angeles, Calif.).

Triplicate cultures were exposed to each hadacidin level alone, to each radiation level alone, and to each combination of hadacidin and radiation. The mean of at least 10 control cultures, exposed to neither hadacidin nor radiation, was used to determine the surviving fractions of cells in treated cultures in each experiment.

The statistical significance of observed differences between test cultures and the irradiated control cultures, corrected for hadacidin toxicity, was determined in the following manner: (1) for convenience in treating large numbers of experiments, the standard deviations of control and treated groups were estimated from the respective ranges and averaged rather than a pooled standard deviation being computed; since the variances of the control and treated groups were nearly the same, this simplification of procedure for applying "Student's" t test had little effect on the numerical result; (2) t was calculated according to the formula

$$t = d/S_{av}\sqrt{(n_1+n_2)/n_1n_2}$$

where d = difference between control and treated means, S_{av} = average standard deviation, n_1 = number of control cultures, and n_2 = number of treated cultures; and (3) p (the approximate probability that the observed difference was due to chance) was obtained from the table of "Student's" t using $n_1 + n_2 - 2$ as the degrees of freedom.

Results and discussion. Using the procedures described above, we have observed that hadacidin potentiates the lethal action of radiation on a number of different mammalian tumor cell lines growing in culture. In Fig. 1-3 are plotted the surviving fractions of Ca755 cells exposed to varying amounts of hadacidin and increasing doses of radiation. The data in Fig. 1-3 illustrate several pertinent points, in addition to their statistical significance: (1) there is a consistent dose response to increasing concentrations of either hadacidin or X-irradiation, and (2) the degree of potentiation of the lethal effects of X-irradiation by hadacidin was dependent upon the dose of X-irradiation, potentiation being absent at nonlethal doses of X-ray and increasing as the X-ray dose was increased. Similarly, the degree of potentiation at a given X-ray dose of approximately the LD_{50} or greater was dependent upon the dose of hadacidin.

Data plots similar in appearance and statistical significance to Fig. 1-3 indicating potentiation of X-irradiation by hadacidin have been obtained with KB and HeLa (S-3) cells. Similarly collected data with H.Ep-2 cells show greater variability and less statistical significance, and hadacidin failed to potentiate the lethal action of X-irradiation on Sa180 cells.

The response of KB, HeLa (S-3), H.Ep-2, and Sa180 cells in culture to hadacidin plus approximately the LD_{90} of X-irradiation are graphically shown in Fig. 4-9. In these figures the fraction of cells surviving in the radiation control (1.0) was corrected for hadacidin toxicity. The data in Fig. 4 were taken from Fig. 1-3 and serve to illustrate the method of presentation of the data in Fig. 4-9.

Increasing the susceptibility of tumor cells (specifically) to the lethal effects of ionizing radiation by combination with potentiating chemicals, if attainable, could extend the practical application of radiation therapy to malignant tumors. Numerous reports of the sensitization of bacterial and mammalian cells in culture to ionizing radiation by pre-irradiation exposure to various halogenated pyrimidines have appeared in recent years(13,14, 15). Attempts to demonstrate chemical potentiation of radiation sensitivity with halogenated pyrimidines in tumor-bearing animals have yielded suggestive but equivocal evidence of chemical sensitization(13,16). The observation that actinomycin D potentiates the lethal effect of ionizing irradiation on HeLa (S-3)(17) cells in culture and the radiation-sensitive Wilms' tumor(18) in man indicates the possibility of positive correlation between *in vitro* and *in vivo* results. The practical need of chemicals which might selectively sensitize tumor cells to the lethal effects of ionizing radiation suggests that searches for new classes of radiation sensitizing compounds should be actively conducted and significant new leads broadly investigated.

Summary. The lethal effects of ionizing

Effect of Hadacidin on X-Irradiated Mammalian Cells

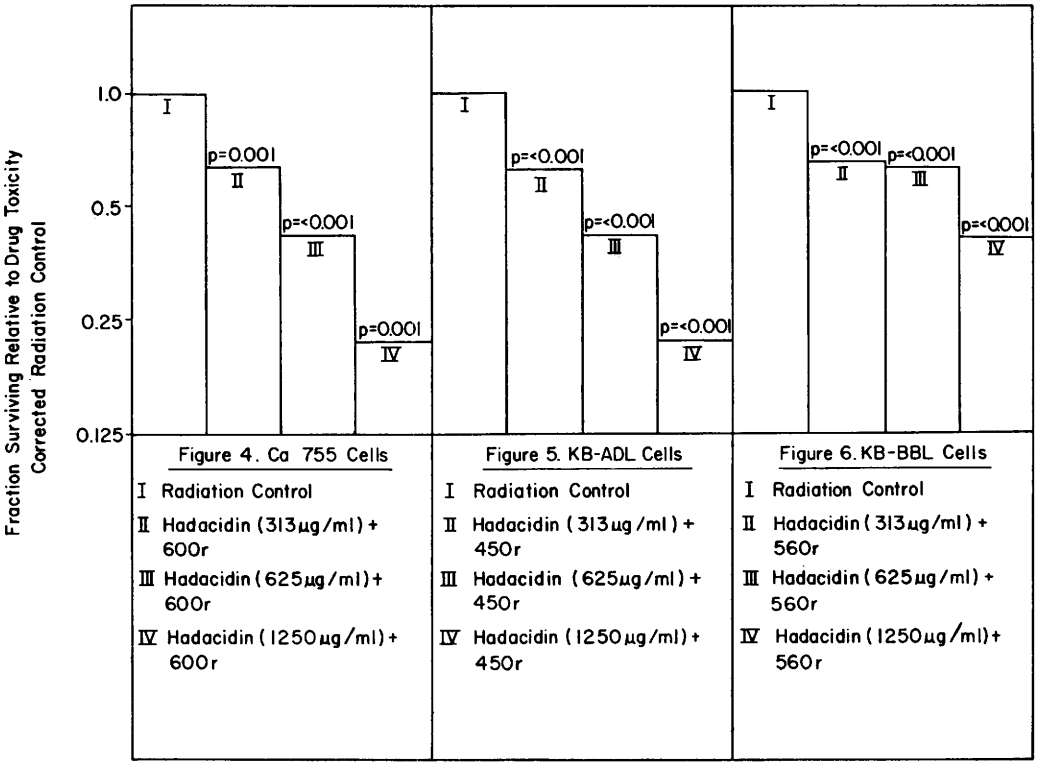

Figure 4. Ca 755 Cells

I Radiation Control

II Hadacidin (313µg/ml) + 600r

III Hadacidin (625µg/ml) + 600r

IV Hadacidin (1250µg/ml) + 600r

Figure 5. KB-ADL Cells

I Radiation Control

II Hadacidin (313µg/ml) + 450r

III Hadacidin (625µg/ml) + 450r

IV Hadacidin (1250µg/ml) + 450r

Figure 6. KB-BBL Cells

I Radiation Control

II Hadacidin (313µg/ml) + 560r

III Hadacidin (625µg/ml) + 560r

IV Hadacidin (1250µg/ml) + 560r

Effect of Hadacidin on X-Irradiated Mammalian Cells

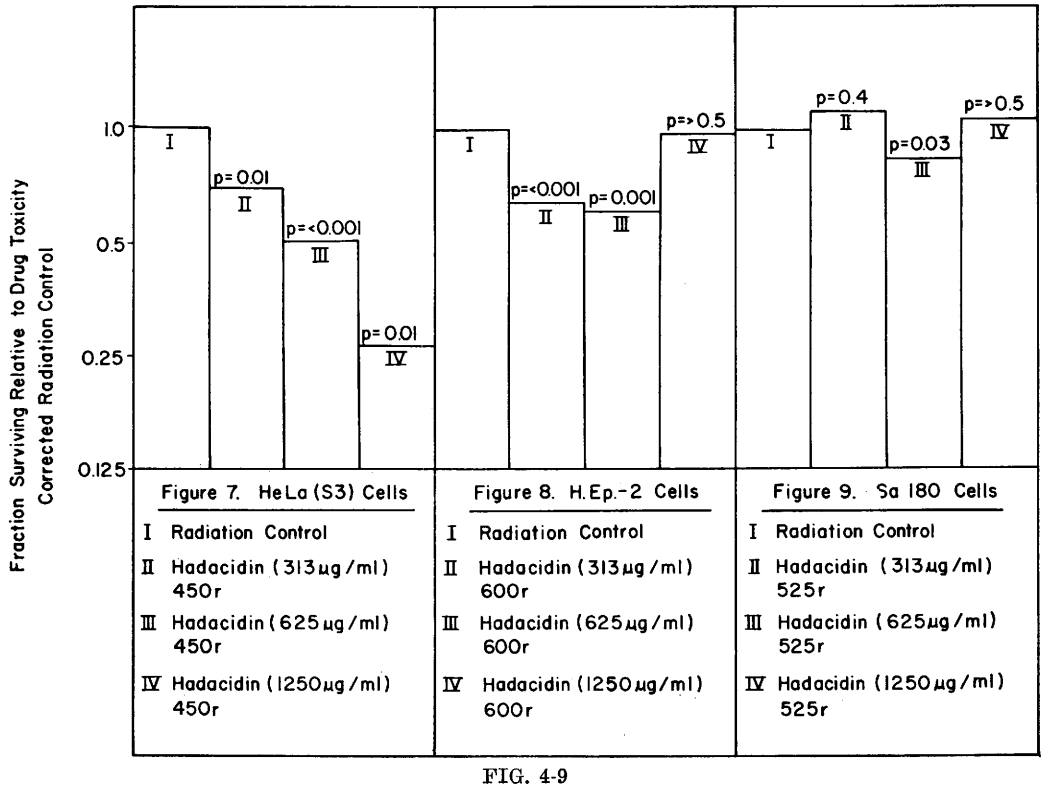

Figure 7. HeLa (S3) Cells

I Radiation Control

II Hadacidin (313µg/ml) 450r

III Hadacidin (625µg/ml) 450r

IV Hadacidin (1250µg/ml) 450r

Figure 8. H.Ep.-2 Cells

I Radiation Control

II Hadacidin (313µg/ml) 600r

III Hadacidin (625µg/ml) 600r

IV Hadacidin (1250µg/ml) 600r

Figure 9. Sa 180 Cells

I Radiation Control

II Hadacidin (313µg/ml) 525r

III Hadacidin (625µg/ml) 525r

IV Hadacidin (1250µg/ml) 525r

FIG. 4-9

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.

We thank Dr. William S. Wilcox for his help with the statistical analysis of the data presented.

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Received October 26, 1964. P.S.E.B.M., 1965, v118.

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.

* This study was supported by the Cancer Chemotherapy National Service Center, Nat. Cancer Inst., under NIH Contract PH-43-62-485.

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