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Hormonal Augmentation of Antimetabolite Chemotherapy.* (25411)

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Our emphasis has been placed on utilizing combinations of drugs in treatment of neoplastic growth(1-5). Previous reports have shown mouse mammary adenocarcinoma 755 to be adversely affected by stilbestrol(5), and by the niacin antagonist, 6-aminonicotinamide (3). The present studies demonstrate the effects of the combined administration of the hormone and the anti-vitamin on the 755 tumor.

Materials and methods. Mammary adenocarcinoma 755 grown in the C57 Bl mouse was the tumor-host system used in all studies. The tumor was implanted into the right axillary region of the mouse by the usual trocar technic. The animals were 2-4 months old, weighed 18-22 g and were housed in plastic cages in an air-conditioned, constant temperature room (74°F). They had free access to Rockland pellets and water. 6-Aminonicotinamide (6-AN), in saline, was administered intraperitoneally at a dose of 2 mg/kg/day; stilbestrol and testosterone, in sesame oil, were administered intramuscularly at doses of 10 mg/kg/day and 25 mg/kg/day respectively. *Biological.* Treatment was initiated on very well-established tumors (14-20 days old) and was continued for 5 to 7 days. In order to note any delayed host toxicity, a waiting period of 5 to 6 days of no treatment was observed after last injection. At the end of this period the animals were sacrificed, the tumors were removed and wet weights were determined to the nearest milligram. A group of animals (labelled "sacrificed controls") was sacrificed on the day that treatment of the remaining groups was initiated. Mean tumor weight of this group served as a reference point for evaluating the effect of chemotherapy on other groups in the experiment. The statistical method used to calculate the significance of the results has been reported(3). *Enzymatic.* The animals were sacrificed by cervical rupture 24 hours after cessation of therapy. The tissues were immediately removed, chilled in crushed ice, blotted dry, and homogenized in 0.25 M sucrose containing 0.02 M nicotinamide and 0.004 M versene in a glass homogenizer of the Potter type(6). The ability of tissue homogenates to convert to citrate was measured as follows: pyruvate, 0.011 M; ATP, 0.007 M; $MgCl_2$, 0.008 M; nicotinamide, 0.04 M; potassium phosphate buffer (pH 7.4), 0.001 M; fluorocitrate, 2.15×10^{-4} M; and the appropriate volume of tissue homogenate was added to ice-cold reaction vessels. Ice-cold isotonic KCl was added to give, after addition of substrate, a final volume of 2.7 ml. Immediately before the vessels were placed in a constant-temperature water bath, 0.2 ml of a 0.10 M malate solution was added. All reactants were neutralized with KOH prior to addition. Incubation was carried out at 37°C with agitation for 30 minutes, at which time the reaction was stopped by addition of 0.5 ml of 30 trichloro-

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TABLE I. Effect of Androgen-Estrogen Therapy on 6-Aminonicotinamide Carcinostasis.

Exp.	Sex	Group*	Mean tumor wt ± S.E. (mg)	No. animals dead/total	% change in body wt
1	♂	Sac. control	463 ± 64		
		6-AN	1009 ± 159	0/20	− 3
		" + Test.	639 ± 129	1/20	+ 8
		Test. + Stilb.	2632 ± 294	1/20	+20
		6-AN + "	526 ± 133	5/20	− 6
		" + Test. + Stilb.	207 ± 45	0/20	− 5
2	♀	Sac. control	405 ± 57		
		6-AN	798 ± 157	0/15	0
		" + Test.	430 ± 105	0/17	+ 7
		Test. + Stilb.	2179 ± 114	0/18	+24
		6-AN + "	300 ± 94	1/16	− 6
		" + Test. + Stilb.	188 ± 46	0/16	0

* Sac. control = Animals sacrificed on day injections begun to other groups. Therapy was begun upon well-established tumors (14-20 days old). 6-AN = 6-aminonicotinamide (2 mg/kg daily); Test. = Testosterone (25 mg/kg daily); Stilb. = Stilbestrol (10 mg/kg daily).

acetic acid (TCA). Citrate analyses(7) were carried out on the supernatant material obtained upon centrifugation. 100 mg of tumor tissue, as homogenate, was added to each reaction flask.

Results. Biological studies. The results of 2 representative experiments are noted in Table I. The chart graphically summarizes all 8 experiments.

The combination of 6-AN and stilbestrol produced a significantly greater anti-tumor effect than either agent administered alone in 3 out of 4 experiments in males and in 3 out of 4 experiments in females. The 4th experiment in males showed borderline significance; the 4th experiment in females was negative.

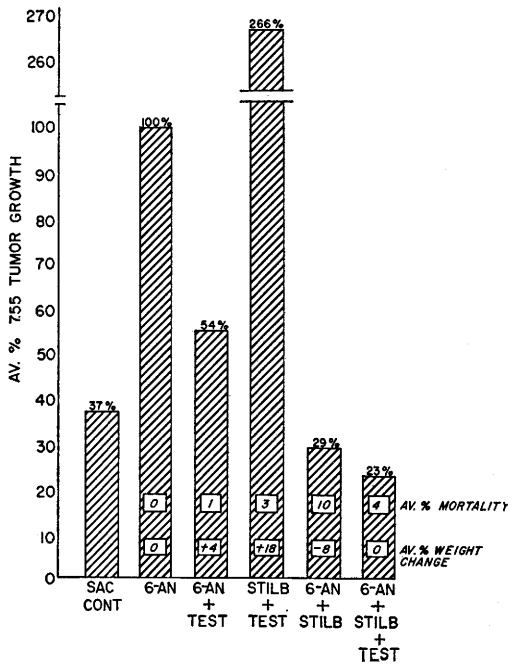

FIG. 1. Combination chemotherapy: 6-aminonicotinamide (6-AN) + stilbestrol (stilb) + testosterone (test). (Summary of 8 experiments, 4 ♂ + 4 ♀.)

TABLE II. Effect of Diethylstilbestrol and 6-aminonicotinamide Administration on Conversion of Malate to Citrate in 755 Tumor Homogenates.

Exp.	Treatment*	Activity,† μmoles citrate produced/30 min./g tissue wet wt
1	Saline control	8.4 ± .4 (9)
	Stilbestrol	8.3 ± .4 (10)
2	6-AN	8.5 ± .3 (8)
	" + stilbestrol	4.7 ± .4 (8)

* 6-aminonicotinamide was administered at level of 2 mg/kg for 6 consecutive days. Stilbestrol was given in sesame oil at 10 mg/kg/day.

† ± stand. error. Figures in parentheses represent No. of tumors analysed.

Addition of testosterone to 6-AN + stilbestrol maintained the anti-tumor effect of the combination of 2 drugs and at the same time markedly reduced host toxicity.

Addition of testosterone to 6-AN resulted in increased carcinostasis over that produced by 6-AN alone. Degree of carcinostasis, however, was not of a statistically significant magnitude in females and only of borderline significance in males.

Biochemical studies. Stilbestrol administration *per se* had no effect on ability of 755 tumor homogenates to convert malate to citrate (Table II, Exp. 1). Stilbestrol, however, markedly enhanced ability of the niacin an-

tagonist, 6-AN, to antagonize conversion of malate to citrate (Table II, Exp. 2). This level of 6-AN (2 mg/kg), by itself, has been shown to have no effect on the malate to citrate pathway(8). Previously, lethal levels of 6-AN were necessary to demonstrate a significant antagonism of the malate to citrate system in 755 tumor homogenates(8). Thus, stilbestrol, by reasons unknown, increases 6-AN toxicity to the 755 tumor without exerting the same degree of augmentation of toxicity to the host tissue. These preliminary enzymatic studies are correlated with the biological findings (Table I). How these findings are related to those of Ville *et al.*(9) and Hurlock and Talalay(10), who have previously shown a relationship between estrogens and pyridine nucleotide metabolism, is unknown.

Summary. 1) The data show that combination of hormone, stilbestrol, and anti-vit. 6-aminonicotinamide, has a greater anti-tumor effect than that produced by either drug alone. Further, addition of testosterone to the combination lessens host toxicity without altering the anti-tumor effect. 2) At an enzymatic level stilbestrol enhanced ability of this niacin antagonist to antagonize conversion of malate to citrate, suggesting a possible mechanism of action to explain the observed biological facts.

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Reversible Inhibition of 2,5-Alkyl Benzimidazoles on Chicken Sperm.* (25412)

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Alkyl benzimidazoles are known inhibitors of a variety of biological systems exhibiting a characteristic order of inhibition(1-6). Because of the nature of inhibition of virus multiplication and the characteristic inhibitory order, it has been suggested that the benzimidazoles inhibit a basic biosynthetic mechanism(s) involving nucleic acids and/or nucleoprotein(2,3,7). With these suggestions in mind, studies presented here were conducted to determine the effect of three 2,5-alkyl benzimidazoles on chicken sperm, a relatively simple biological system whose metabolic processes were assumed to involve mostly respiration.

Materials and methods. Pooled samples of semen were used. Semen was collected from Univ. of Maryland flightless strain males by massage technic. 2,5-Dimethylbenzimidazole (DMB) and 2-ethyl-5-methylbenzimidazole (EMB) were dissolved in 0.07 N HCl and 2-hepta-5-methylbenzimidazole (HMB) was

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