Fluorouracil Inhibition of Testosterone-Stimulated Growth of Seminal Vesicles.* (29113)

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A previous study(1) of the inhibitory effect of 5-fluorouracil (5-FU) on hormone-stimulated growth of specific target tissues suggested the desirability of a more systematic investigation of the effects of graded doses of 5-FU at different levels of growth stimulation. The system selected for this purpose was the seminal vesicle of the castrate rat, stimulated by testosterone propionate.

Materials and methods. Adult (150 g) male Sprague-Dawley rats (Barkbridge Farms, N. J.) were castrated. Three weeks later the animals were divided into 3 groups of 24 each, treated as follows: *Group 1.* Testosterone propionate (T.P.) in oil, subcutaneously, daily. *Group 2.* Testosterone propionate in oil, subcutaneously, daily, and 5-FU intraperitoneally, daily, the first injection of the latter given on the day preceding the first testosterone injection. *Group 3.* No treatment.

Testosterone propionate was given in dosages ranging from 0.1 to 100 mg daily and 5-FU in dosages ranging from 6.25 to 25 mg/kg/day, for periods of 3 to 10 days (Table I).

All animals were fed laboratory chow *ad libitum* and had free access to water. They were sacrificed by exsanguination under light ether anesthesia 24 hours after the last treatment. Seminal vesicles were weighed immediately after removing the contents by blotting. An aliquot was dried to constant weight in an oven at 100°C for dry weight measurement.

Results and discussion. The average wet weight of the seminal vesicles in the control animals ranged from 48.6 mg ± 1.64 (Standard Error) in the 3-day treatment group to 45.7 mg ± 1.84 in the 10-day group. The dry weight:wet weight ratio (× 100) averaged 23.4 ± 2.26 for all control animals. As has been reported elsewhere(1), the weight increment induced by testosterone was associated with a higher water content, *i.e.*, decreased percentage dry weight (average 15.0 ± 1.0). This hydration effect of testosterone was not significantly altered by simultaneous administration of 5-FU, despite the striking inhibition of growth that occurred at certain dosage levels(1).

The effect of 5-FU on the seminal vesicles under the various treatment regimens is indicated by the data in the Table. Increase of either hormone dosage or duration of treatment resulted in diminished inhibition of testosterone-induced growth by 5-FU at all dosage levels of the antimetabolite. This relationship was reflected also in the absolute values for seminal vesicle weight; *e.g.*, with 3 days of treatment the weight increment produced by 1 mg/day of T.P. plus 6.25 mg/kg/day of 5-FU approximated that produced by 10 mg of hormone plus 12.5 or 25 mg/kg of 5-FU (290 and 230%, respectively). This is particularly interesting in view of the fact that the growth of the seminal vesicles induced in 3 days by 1 mg of T.P. did not differ materially from that induced by 10 mg (310 and 360%, respectively). Moreover, the growth inhibitory effect of the maximum tolerated dose of 5-FU (25 mg/kg/day) was completely overcome by increasing the amount of androgen.

Although the effects of hormones and antimetabolites on growth processes have been studied extensively, there is little available information concerning interactions of these factors. Gelfant *et al*(2) investigated the effects of aminopterin and nitrogen mustard on estrogen-induced growth of the uterus; mitotic activity was reduced markedly, whereas the increase in tissue mass was only moderately depressed. In a previous study(1) we found that 5-FU inhibited the growth-stimulating effect of partial hepatectomy

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TABLE I. Effect of 5-Fluorouracil on Testosterone-Induced Growth of Castrate Seminal Vesicle.

		3 days			7 days			10 days		
Testosterone propionate	Fluorouracil	No.	% increase dry wt	% inhibition	No.	% increase dry wt	% inhibition	No.	% increase dry wt	% inhibition
(mg/day)	(mg/kg/day)									
.1	0	24	220	—	24	520	—	24	720	—
"	6.25	24	150	32*	24	490	N.S.‡	20	690	N.S.
"	12.5	24	80	64†	24	340	35†	18	660	N.S.
"	25	20	80	64†	18	310	40†	—	—	—
1.0	0	24	310	—	24	840	—			
"	6.25	24	290	N.S.	24	820	N.S.			
"	12.5	22	200	32†	20	790	N.S.			
"	25	22	170	45†	16	670	20*			
10	0	24	360	—	24	910	—			
"	6.25	24	340	N.S.	22	900	N.S.			
"	12.5	24	290	19*	20	870	N.S.			
"	25	20	230	36†	16	840	N.S.			
100	0	24	680	—						
"	12.5	24	610	N.S.						
"	25	22	630	N.S.						

* "P" value (by Fisher's "Student" method) of difference against T.P.-treated <.05.
† "P" value <.01.
‡ N.S. = not significant.

(liver), somatotropin (epiphyseal cartilage), and testosterone (seminal vesicle), the influence on mitotic activity being greater than on dry weight (liver, seminal vesicle).

The principal primary effect of 5-FU in mammalian cells is inhibition of DNA synthesis. Although this can occur at low dosage levels without demonstrable depression of protein synthesis(3,4), there is evidence that with larger doses this primary effect is followed by decreases in rates of synthesis of nuclear and cytoplasmic RNA and protein (5). The inhibitory influence of 5-FU on growth stimulation by testosterone is readily understandable on this basis. However, the converse is not so readily explicable, *i.e.*, the effect of increasing dosage of testosterone in overcoming growth inhibition by 5-FU.

Castration is followed by progressive decrease in weight and in total DNA, RNA and protein of the seminal vesicles, with decrease in both size and number of cells(6,7). These changes are reversed by administration of testosterone, which causes increased mitotic activity(7) and relatively greater increase in RNA than in protein(8). On the basis of *in vitro* studies, Wilson(9) concluded that enhancement of protein synthesis in the seminal vesicles by testosterone is due primarily to acceleration of conversion of sRNA-amino acid complexes to microsomal ribonucleoprotein. Other observations(10) are consistent with the view that androgen governs the level of template RNA attached to ribonucleoprotein particles in accessory sex tissues. It is difficult to understand how stimulation of this mechanism could effectively overcome the effects on growth of the more fundamental block in DNA synthesis produced by large doses of 5-FU.

Uracil is incorporated into RNA by way of uridine and uridylic acid, and the latter into DNA through formation of deoxyuridine monophosphate, which undergoes methylation to thymidylic acid. The major growth-inhibitory influence of 5-FU, which also follows these metabolic pathways(11), is due to block of the methylation reaction. Administration of testosterone causes increased incorporation of uracil (and, presumably, of 5-FU) into RNA of target tissues(12). It is possible that stimulation of this pathway in animals receiving testosterone and 5-FU may occur at the expense of formation of fluorodeoxyuridine monophosphate, with consequent decreased inhibition of DNA synthesis. We have preliminary evidence that this is the case.

The observations reported here indicate that the effectiveness of an antimetabolite in inhibiting a normal growth process may be altered by changes in the intensity of the growth stimulus. A similar principle may conceivably apply to neoplastic growth. If so, there are implications with respect to possible benefits of combination therapy in certain types of tumors.

Summary. The growth-stimulating effect of testosterone on the seminal vesicle of the castrated rat is inhibited by 5-fluorouracil. The extent of this inhibition diminishes with increase of either hormone dosage or duration of treatment, and may be overcome completely by appropriate amounts of hormone at all tolerated levels of fluorouracil administration.

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Nature of Intestinal Phytase Activity.* (29114)

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Phytases, enzymes hydrolyzing phytic acid, are widely distributed in animal tissues. Phytase activity was first observed in the intestinal mucosa of rats(1) and later in the chicken, pig and cow(2). Phytase activity has been demonstrated to be widely distributed in the rat, the highest activity being found in the proximal part of the intestinal mucosa(3).

An increase has been reported in the intestinal phytase activity of rats and chicks kept on cereal rachitogenic rations when supplemented with vitamin D(4). Pilleggi *et al* (5) have observed identical responses in phytase and alkaline phosphatase activities in intestinal extracts of rats fed Vit. D. However, no consistent correlation, in a quantitative sense, between degree of phytate hydrolysis by the intestinal homogenates *in vitro* and calcification could be found(6). This suggested that a non-specific phosphatase may act on the phosphate groups of phytates. In the present work, the effect of feeding dicalcium phosphate and sodium and calcium phytates on intestinal phytase activity in rats, chicks and hens and the effect of fluoride on intestinal phytase and alkaline phosphatase activities has been studied. The results of fluoride inhibition and mixed substrates on phytase and alkaline phosphatase activities were used to examine the enzyme(s) involved(7).

Methods and materials. Dietary phosphorus levels in the range of 0.2-0.6% phosphorus, supplied as dicalcium phosphate, were fed to day-old chicks, mature hens and weanling rats for experimental periods of 4, 2 and 6 weeks, respectively. In addition, either sodium or calcium phytate was fed at levels

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