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Effects of Growth Inhibitors on Response of Rat's Uterus to Estrogen.* (19247)

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Hertz and Sebrell(1) first reported the failure of estrogens to induce growth of the female reproductive tract in the absence of folic acid in the chick, and subsequent studies followed on the frog(2), rat(3), and monkey (4,5). In all of these studies the main objective was centered around gross morphological observations and weight relationships. The experiments reported here represent an attempt to examine the physiological nature of the inhibitory process produced by folic acid antagonists on the growth response of the uterus to estrogen, and to determine in what way this may differ from similar inhibitory effects produced by other compounds such as beryllium(6), cadmium(7), and strychnine. These comparisons are made on the basis of inhibitory effects on the imbibition of water by the uterine tissue in response to estrogen and changes in nitrogen, fat, and glycogen content.

Materials and methods. A total of 56 100-day-old female albino rats, of an inbred strain developed from Wistar stock, weighing 175-200 g, were used for these experiments. A folic acid antagonist (Aminopterin†), and beryllium, cadmium, and strychnine were given concurrently with estradiol.‡ The estradiol was dissolved in sesame oil, and the other drugs in physiological saline. All injections were made subcutaneously and the dosage of beryllium, cadmium, and strychnine was such that the animals given such treatment would survive for at least two weeks without loss of weight. The animals given aminopterin for 3 to 4 days lost little or no weight. Nitrogen determinations were made colorimetrically(8), while the tests for fat and glycogen were made by histochemical

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TABLE I. Effect of a Folic Acid Antagonist and Beryllium, Cadmium, and Strychnine on Water Content and Total Nitrogen of Uteri of Ovariectomized Rats Given Estrogen.

Group	Treatment	Uterine wt, mg	% H_2O	N, μg/mg
I	——	89.7	79.6	23.2
II	.1 μg est.*	190	80.9	24.5
III	.1 μg est. + 50 μg A5040†	129.3	78.4	22.5
IV	.1 μg est. + 50 μg A9332	132.7	78	23.4
V	.1 μg est. + 30 μg A5801	162	80.3	21.7
VI	.1 μg est. + .1 mg Be./g B.W.‡	167.8	77.8	23.5
VII	.1 μg est. + .01 mg Cd./g B.W.	147.6	79.7	24.2
VIII	.1 μg est. + 1 mg strychnine/kg B.W.	129.1	78.9	25.6

* Estradiol-17-β. † A = Aminopterin (4-aminopteroylglutamic acid). ‡ B.W. = Body wt.

procedures(9,10). The animals were ovariectomized and divided into 8 groups at the very beginning of these experiments. The animals of Group I served as castration controls, and were killed 11 days after the operation. The Group II animals, 7 days after castration, were given 0.1 μg estradiol daily for 3 days. The remaining groups, III to VIII, were put on experiment after the uteri had undergone one week of castration atrophy. On the seventh day of this period a single dose of the inhibitor was given, and the treatment was continued for 3 additional days during which both the inhibitor and estradiol were given concurrently. The animals were killed 24 hours after the last injection. The uteri were removed and slit lengthwise and blotted on bibulous paper thus removing excess fluid, and weighed separately on a Roller-Smith torsion balance.

Results and discussion. All animals treated with aminopterin, beryllium, cadmium, and strychnine showed uteri that were much smaller than those given estradiol alone. There was also a decrease in percentage of nitrogen in the uteri of those receiving the folic acid inhibitor. Aminopterin 5040 and 9332, in equivalent doses, produced a decrease of 8.1 and 4.5% respectively, while 5801, which was more toxic, produced a decrease of 11.4% at three-fifths the dose of the other two. This is in sharp contrast with the results for controls given estrogen alone. In these there was an increase of 5.6% in uterine nitrogen (Table I).

A decrease in the total nitrogen content of the uteri was also obtained in those animals receiving beryllium and cadmium, 4.1 and 1.2% respectively. On the other hand, strychnine did not effect a decrease in the nitrogen content of the uterus, but on the contrary was associated with an increase of 4.3%. Therefore, strychnine has a very weak inhibitory action on the effect of estrogen.

The only animals that showed a positive test for fat were the untreated castrate controls. This is in agreement with the observations of Dietlein(11) who has found that estrogen does not only fail to bring about the deposition of fat in the endometrium of the rat's uterus but removes any that may be present in the luminal epithelium. Thus, it appears that in these experiments sufficient estrogen remained not inhibited to remove the fat that was presumably present in the luminal epithelium of the castrated animal.

Glycogen was absent from the uteri of both experimental and control animals. This was checked both by colorimetric(12) and histochemical methods(10). Likewise, there was a uniformity of results with regard to the imbibition of water by the uterus in that each inhibitor produced a decrease as compared with controls given estrogen alone.

Summary. Aminopterin, beryllium, cadmium, and strychnine proved effective in reducing the physiological activity of estradiol. All compounds except strychnine effected a decrease in total nitrogen content in the uteri of ovariectomized rats receiving estradiol. Such inhibition was associated with a decrease of water in the uterine tissue and the absence of fat and glycogen. Thus, the inhibitory process is associated with these physiological changes.

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An Influence of 2,6-Diaminopurine upon the Content of Kappa in *Paramecium aurelia,* Variety 4.* (19248)

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Many compounds which, from their structure, might be expected to interfere with nucleic acid metabolism, have been tested for their ability to restrain the growth of neoplasms in experimental animals(1-7). The studies are based upon an assumption that the metabolism, the pattern of uptake of precursors of nucleic acid in normal cells, is different from that in neoplastic ones. It is assumed, furthermore, that the differences are sufficiently important to permit their use in inducing by anti-metabolites the selective injury of the neoplastic cell type, as compared with the normal. This approach to cancer chemotherapy has the advantage, in theory, of being applicable whether the neoplastic process be due to a virus or to a mutated genic structure, since both are dependent for function on their component nucleic acids. Of the compounds which have been synthesized and tested as potential antimetabolites of nucleic acid, few have exerted a high degree of growth restraint of any type of neoplasm *in vivo.* None have been curative. Nevertheless, *in vitro,* a substantial degree of selective injury to some, but not all, neoplastic cells, as compared with normal types growing at the same rate and of generally similar origin, has been demonstrable (8).

The possibility of differential toxicity based upon the presumed specificity of nucleic acid metabolism of different type cells is attractive and important. Accordingly, new means were sought to provide evidence pertinent to this assumption. The characteristics of the cytoplasmic factor kappa in the ciliated protozoan, *Paramecium aurelia,* as elucidated by Sonneborn and his associates(9-18), indicated that it might provide a useful subject for study. Certain stocks of *P. aurelia,* known as killers, contain a sufficient concentration of kappa particles to produce paramecin, a substance lethal to other stocks of the protozoan. The susceptible animals, known as sensitives, contain no kappa particles and form no paramecin. X-ray inactivation studies and staining procedures indicate that the kappa particle is roughly comparable in size to certain viruses and rickettsia. Kappa particles are self-duplicating at rates independent of the fission rate of the paramecia containing them, and contain desoxyribonucleoprotein(15,16), as does their product, paramecin(17). Could kappa, an easily demonstrable and functionally unique cell constituent, be destroyed with-

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