TECHNICAL PAPERS

Inhibition of Estrogen-induced Growth in the Genital Tract of the Female Chick by a Purine Antagonist; Reversal by Adenine

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We have previously reported: (a) that folic acid is required for optimal tissue growth response to estrogen in the genital tract of the female chick and monkey; (b) that several folic acid antagonists quantitatively inhibit estrogen-induced growth in the genital tract of the female chick and rat; and (c) that such inhibition is reversed by the addition of high doses of folic acid (*2, 3, 4*).

The observation of Stokes (*9*) that thymine can replace folic acid as a growth requirement for certain lactobacilli initially suggested that folic acid is involved in purine and pyrimidine metabolism. Accordingly, Hitchings, *et al.* have prepared a number of purine and pyrimidine derivatives which exhibit antifolic acid activity when tested on bacteria. This inhibitory effect is reversible by adenine, i.e., 6-aminopurine, and related compounds (*6, 7*).

TABLE 1

EFFECT OF 2,6-DIAMINOPURINE ON ESTROGEN RESPONSE IN CHICK GENITAL TRACT*

Group	Stilbestrol	2,6-diamino purine (mg)	Adenine sulfate (mg)	Number of chicks	Oviduct weight (mg)
I	–	..	.	10	8 ± 3
II	+	..	..	10	50 ± 5
III	+	15	..	10	14 ± 5
IV	+	10	..	10	22 ± 3
V	+	10	4	10	30 ± 5
VI	+	10	8	10	37 ± 6
VII	+	10	50	8	42 ± 4

* Day-old N. H. red chicks were used; they were given no food but water *ad libitum*; Stilbestrol given at 1 mg daily subcutaneously in 0.2 cc corn oil for 2 days. Other compounds injected at indicated daily dose for 3 days in 1.0 cc aqueous solution or suspension, except for group VII which received adenine by capsule; all chicks were autopsied 24 hr after the last injection.

We wish to report that one of the purine analogues, 2,6-diaminopurine,[1] exerts a marked inhibitory effect upon the estrogen response in the genital tract of the female chick and that this inhibition is largely reversible by adenine, i.e., 6-aminopurine. Table 1 presents a representative experimental series.

It is particularly noteworthy that as much as 5 mg of folic acid does not reverse the inhibition produced by 10 mg of 2,6-diaminopurine (*5*).

In view of our earlier observations concerning the critical role of folic acid in the tissue growth response to estrogens (*2–4*), the present data support the view that folic acid may be concerned with purine and pyrimidine metabolism (*9*).

In any event, it is apparent that folic acid and adenine, and their respective inhibitory analogues, can quantitatively determine the degree of response obtained in a tissue which is under maximal hormonal stimulus for growth. These observations should provide a useful experimental tool for the further study of the basic metabolic mechanisms involved in growth processes in hormone-sensitive tissues.

Moreover, these phenomena may provide a basis for the development of chemical agents of therapeutic value in such clinical states as prostatic and breast cancer, in which a suppression of the biological effectiveness of endogenous steroid hormones has proven beneficial (*1, 8*).

[1] We are very much indebted to Dr. G. H. Hitchings, Wellcome Research Laboratories, for making this and related compounds available to us; without his generous cooperation these investigations would have been impossible.

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A Capillary-Ascent Test Tube Method for Separating Amino Acids by Filter Paper Chromatography[1]

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Williams and Kirby's (*3*) capillary-ascent modification of the method of Consden *et al.* (*1*) for separating amino acids by filter paper chromatography has been adapted to the separation of less than microgram quantities of amino acids in 6-in. test tubes. It has been shown that amino

[1] Paper 54. For Paper 53, see Dunn *et al.* (*2*). This work has been aided by a grant from the National Institutes of Health (U. S. Public Health Service).