TECHNICAL PAPERS

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

Roy Hertz and Wm. W. Tullner

Endocrinology Section, National Cancer Institute, Bethesda

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.

References

1. BOYD, S. *Brit. med. J.*, 1900, **2**, 1161.
2. HERTZ, R. *Endocrinology*, 1945, **37**, 1.
3. ———. *Proc. Soc. exp. biol. Med.*, 1948, **67**, 113.
4. ———. *Science*, 1948, **107**, 300.
5. ——— and TULLNER, WM. W. Unpublished data.
6. HITCHINGS, G. H., ELION, G. B., and VANDERWERFF, H. *Fed. Proc.*, 1948, **7**, 160.
7. HITCHINGS, G. H., ELION, G. B., VANDERWERFF, H., and FALCO, E. A. *J. biol. Chem.*, 1948, **174**, 765.
8. HUGGINS, C., STEVENS, R. E., and HODGES, C. V. *Arch. Surg.*, 1941, **43**, 209.
9. STOKES, J. L. *J. Bact.*, 1944, **48**, 201.

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

Louis B. Rockland and Max S. Dunn

Department of Chemistry, University of California, Los Angeles

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).

acids may be separated conveniently, rapidly, and economically by the described procedure.

Strips[2] (13.5 × 1.80 × 1.00 cm) were cut with a paper

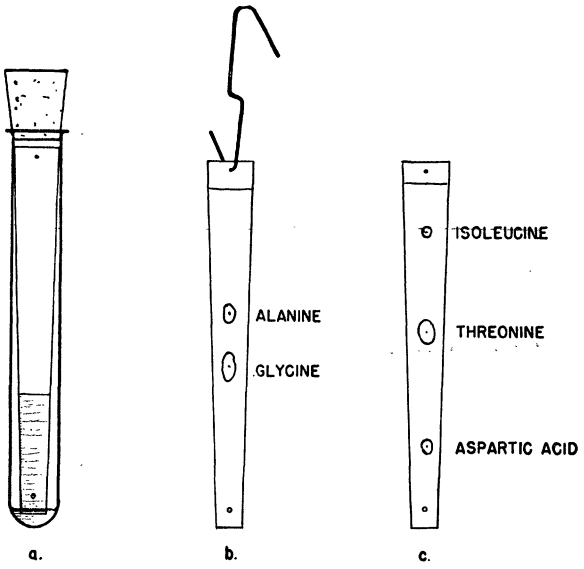

FIG. 1.

cutter from Whatman's No. 1 filter paper (14 × 19 cm) with the aid of a line-guide sheet[3] placed over and stapled to the narrow end of the filter paper. It was found convenient to prepare replicate strips by cutting a package of six sheets of filter paper enclosed between two sheets of white wrapping paper. Each packet of six strips was pierced in the center and about 4 mm from the broad end to facilitate suspending the strips during the subsequent drying process. Approximately 500 strips were obtained from six sheets (45.7 × 56.3 cm) of filter paper.

The amino acid solution[4] is drawn into a capillary pipet[5] and the tip of the pipet is applied quickly and lightly to the strip at the center and about 6 mm from its narrow end. The wet area, which should not be in excess of 1.5 mm in diameter, is circled and allowed to dry (5 min). Approximately 0.4 ml of water-saturated phenol[6] is pipetted into the bottom of a 6-in. test tube and the narrow end of the amino acid treated strip is inserted into the phenol solution in a position (shown in Fig. 1.a.), such that the strip does not touch the walls of the tube except at the upper end. The tube is stoppered with a good quality, rolled No. 9 cork. The tube is allowed to stand for 2 to 3 hr until the solvent ascends to a position about 5 mm below the perforation in the strip. The strip is removed, suspended on a bent paper clip and dried at 110° for about 3 min. The dried strip is sprayed lightly with a 0.25% solution of ninhydrin in water-saturated butanol, and dried at 110° for about 4 min.

The colored spots observed with transmitted light are circled and the center marked. The distances from the center of the original wet area *A* and the final spots *B* and from the former and the solvent boundary *C* are measured. The R_f values are calculated as the ratios, *AB/AC*. Tracings of typical chromatograms are shown in b and c of Fig. 1. The R_f values for 17 amino acids (shown in Table 1) compare favorably with those obtained by larger scale, one-dimensional paper chromatography.

TABLE 1

R_f VALUES OF AMINO ACIDS WITH PHENOL

Amino acid	Consden *et al.* (*1*)*	Williams and Kirby (*3*)†	Authors†
Alanine	0.59	0.58	0.62
Arginine	0.62	0.54	0.53‡
Aspartic acid	0.17	0.22	0.25
Glutamic acid	0.28	0.23	0.39
Glycine	0.42	0.36	0.49
Histidine	0.69	0.62	0.81‡
Isoleucine	0.86	0.83	0.85
Leucine	0.88	0.80	0.86
Lysine	0.48	0.41	0.41‡
Methionine	0.81	0.74	0.74
Phenylalanine	0.93	0.83	0.87
Proline	0.86	0.88	0.87
Serine	0.36	0.30	0.33
Threonine	0.50	0.43	0.57
Tryptophan	0.86	0.71	0.81
Tyrosine	0.62	0.55	0.53
Valine	0.77	0.72	0.82

* Descending boundary method.

† Ascending boundary method. Time and distances: 28 hr and 380 mm (*3*); 3 hr and 125 mm at about 25° (authors).

‡ Used as monohydrochloride histidine, monohydrochloride monohydrate.

A rapid, convenient, capillary-ascent test tube method has been described for the separation of less than microgram quantities of amino acids by paper chromatography. R_f values, comparable to literature data, have been determined for 17 amino acids. Numerous samples may be determined simultaneously by the authors' method.

[2] The filter paper should not be touched with the hands, to avoid amino acid contamination.

[3] Line-guide sheets may be drawn by hand with the aid of a metal template or, more conveniently, they may be printed by means of a stencil which is cut in such a manner that adjacent strips are inverted.

[4] 0.03 *M* solutions have given satisfactory chromatograms of the amino acids investigated.

[5] Prepared by drawing 5-mm pyrex capillary tubing to about 2 mm and grinding the tip to about 0.5 mm with 400–600 mesh carborundum. About 0.0002 ml of solution is delivered by the described technique. Volumes of solution smaller than 0.0002 ml are delivered by the Gilmont Ultra-Microburet (The Emil Greiner Company, 161 6th Ave., New York City).

[6] Phenol which gives a red-colored solution is preferred since it marks the solvent boundary more clearly than chemically pure material. Pink fronts may be eliminated by treatment with coal gas, ammonia, HCN, or cupron (*1*).

References

1. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*, 1944, **38**, 224.
2. DUNN, M. S., MCCLURE, L. E., and MERRIFIELD, R. B. *J. Biol. Chem.* In press.
3. WILLIAMS, R. J. and KIRBY, H. *Science*, 1948, **107**, 481.