

Citrovorum Factor and Oviduct Response to Stilbestrol in Aminopterin-Treated Chicks. (18436)

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Response of the chick oviduct to stilbestrol is drastically limited by a deficiency of pteroylglutamic acid (PGA), whether the deficiency be produced by feeding a PGA-deficient diet or by administering PGA antagonists(1-4). There is, furthermore, a quantitative relationship between the response of the chick oviduct to injected stilbestrol and the dietary level of PGA or the administered dose of aminopterin, a highly potent antagonist of PGA(2,4). Protection against aminopterin interference with stilbestrol action on the chick oviduct is afforded by PGA administered one hour prior to aminopterin in a ratio of PGA to antagonist of 300:1(2). Failure of high doses of PGA to prevent the toxic effects of aminopterin in several species has been reported(5-9). In mice, however, positive protection occurs with a ratio of PGA to aminopterin of 500:1(10). More efficient than PGA in preventing aminopterin is the factor (CF) essential to *Leuconostoc citrovorum* (11-13,15), a substance which has been

shown to be related to PGA by chemical(13) and metabolic(14) synthesis from PGA. Nichol and Welch(15) found that 250,000 units of CF counteract the toxic effects of 25 μ g of aminopterin in rats deprived of a dietary source of PGA for 7 days; in mice, about 20,000 units of CF were required to overcome 1-4 μ g of aminopterin, according to the work of Broquist *et al.* as reported by Cravens and Snell(12); while for *L. citrovorum* only 11 units of CF were required per 1-4 μ g of aminopterin(16); in the chick embryo, as high as 6 million units partially counteract 20 μ g of aminopterin(12).

The data presented below indicate that, under the experimental conditions employed, CF prevents aminopterin inhibition of stilbestrol stimulation of the chick oviduct in a ratio of vitamin to antagonist of about 16:1. Under the same conditions PGA in a ratio of 500:1 is 81% as effective in preventing aminopterin inhibition.

Materials and methods. Two-day-old White Leghorn pullets obtained from Kerr Chickeries, Frenchtown, N. J., were arranged in 16 groups as indicated in Table I and were given their first injections immediately upon arrival in the laboratory. They were thereafter housed in electrically heated brooders with raised, wire-mesh floors. No food was allowed but tap water was provided *ad libi-*

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TABLE I. Effect of Citrovorum Factor on Aminopterin Inhibition of Stilbestrol Stimulation of the Chick Oviduct.

Group No.	No. of chicks	Injections					Autopsy results		
		PGA total dose, 22.5 mg	CF total dose, μ g	4NH ₂ -PGA, total dose, 15 μ g	Stilbestrol in corn oil, total dose, 2 mg	Corn oil only	No. of survivors	Mean body wt, g $\pm$ S.E.*	Mean oviduct wt, mg $\pm$ S.E.
1	10	0	0	0	0	+	10	36.2 $\pm$ 0.9	11.2 $\pm$ 1.5
2	12	0	0	+	0	+	11	33.5 $\pm$ 0.7	11.5 $\pm$ 0.9
3	10	0	0	0	+	0	9	33.0 $\pm$ 0.9	63.9 $\pm$ 3.5
4	26	0	0	+	+	0	17	33.4 $\pm$ 0.5	23.4 $\pm$ 0.8
5	10	+	0	0	0	+	9	32.1 $\pm$ 0.8	15.9 $\pm$ 1.2
6	10	+	0	+	0	+	9	31.2 $\pm$ 0.9	13.9 $\pm$ 0.9
7	10	+	0	0	+	0	10	31.8 $\pm$ 0.6	73.8 $\pm$ 4.1
8	10	+	0	+	+	0	10	31.7 $\pm$ 0.9	60.4 $\pm$ 2.7
9	10	0	30	0	0	+	9	32.3 $\pm$ 0.8	14.3 $\pm$ 0.8
10	12	0	30	+	0	+	12	32.5 $\pm$ 0.7	12.8 $\pm$ 1.0
11	10	0	30	0	+	0	10	33.0 $\pm$ 1.1	71.4 $\pm$ 4.1
12	20	0	30	+	+	0	20	33.8 $\pm$ 0.5	39.5 $\pm$ 1.8
13	9	0	240	0	0	+	7	30.0 $\pm$ 0.6	12.6 $\pm$ 1.2
14	12	0	240	+	0	+	12	33.5 $\pm$ 0.8	13.4 $\pm$ 0.6
15	10	0	240	0	+	0	10	33.0 $\pm$ 0.5	61.5 $\pm$ 1.6
16	20	0	240	+	+	0	20	35.7 $\pm$ 2.0	55.1 $\pm$ 2.5

* Standard error of the mean.

tum. The complete injection schedule was as follows:

Day	Time	Injections
1	4:00 P. M.	PGA, CF or saline
	5:30	Aminopterin
2 and 3	8:30 A. M.	PGA, CF or saline
	10:00	Aminopterin and Stilbestrol (or oil)
4	8:00-12:00	Autopsy

Dosages were, per dose:

PGA: 7.5 mg in 0.5 ml solution (Folvite¹).
CF¹: 10 μ g or 80 μ g in 0.5 ml of 0.9% saline.
Aminopterin:* 5 μ g in 0.1 ml of 0.9% saline.
Stilbestrol:† 1 mg in 0.1 ml of corn oil.

Controls: 0.5 mg of 0.9% saline was used as the control solution in chicks receiving neither PGA nor CF, while 0.1 ml of corn oil was administered to those receiving no stilbestrol.

At autopsy, the chicks were killed with

*Pteroylglutamic acid (Folvite), aminopterin and Citrovorum Factor Concentrate were generously supplied by the Lederle Laboratories Division of the American Cyanamid Co. through the courtesy of Dr. T. H. Jukes. According to information received from Dr. Jukes the Citrovorum Factor Concentrate (Leucovorin, synthetic Citrovorum Factor) made available to us contained 20 Million Units/ml. Five μ g represent about 25000 Units.

† Stilbestrol was kindly made available by Winthrop-Stearns, Inc.

chloroform and weighed. Oviducts were then immediately dissected out, blotted, and weighed on a Roller-Smith torsion balance to the nearest milligram.

Results. In newly-hatched chicks subsisting on their own body stores† for 5 days, 2 mg of stilbestrol stimulated a 5.5 fold increase in oviduct weight in 48 hours (Table I). Aminopterin (15 μ g total dose) prevented approximately two-thirds of the stilbestrol-induced proliferation but did not reduce the sensitivity of the oviduct to endogenous estrogen.

In PGA-supplemented chicks a significantly greater oviduct growth occurred even though body weight decreased significantly (Tables I and II). This may indicate greater ovi-

TABLE II. Statistical Comparisons of Mean Oviduct Weights.

Groups compared	t*	P
1 and 5	3.1	<0.01
3 " 7	1.85	0.1-0.05
3 " 11	1.25	0.3-0.2
11 " 15	1.96	0.1-0.05
15 " 16	0.51	>0.5
7 " 8	2.85	0.02-0.01

* Fisher's t value(17).

† The caloric value of the injected corn oil was 772 small calories per 0.1 cc oil.

duct sensitivity to, decreased inactivation of or increased secretion of endogenous estrogen. Aminopterin, at the dose employed, did not interfere with the endogenously increased oviduct growth.

When stilbestrol was administered to PGA-supplemented chicks, in the absence of aminopterin treatment, oviduct proliferation was significantly greater than in non-supplemented chicks. The increase in oviduct weight was 6.5 fold in comparison to Group 1 and 4.5 fold with reference to Group 5. Aminopterin, under the conditions used, interfered only to the extent of 19% with the action of stilbestrol in the PGA-treated chicks. That is to say, in the time intervening between the injection of PGA and the injection of aminopterin, the conversion of PGA to its metabolically active form(15) was adequate to permit 81% of the possible stilbestrol action. If aminopterin is also antagonistic to the active form of PGA(15) possibly more conversion occurred than is evident in the data.

Citrovorum factor (CF) in a total dose of

30 μ g permitted growth of the oviduct both in the absence and presence of exogenous estrogen as well as did a total dose of 22.5 mg of PGA but was not as effective in preventing the action of aminopterin (total dose of 15 μ g). When the total dose of CF was increased to 240 μ g, no greater stilbestrol action was permitted than occurred with the lower dose of CF or the massive dose of PGA but the higher dose of CF did effectively prevent aminopterin interference with stilbestrol action on the oviduct.

Summary. Data have been presented which show that Citrovorum Factor (CF) in a ratio to aminopterin of approximately 16:1, but not of 2:1, counteracts aminopterin inhibition of stilbestrol stimulation of the chick oviduct, while pteroylglutamic acid (PGA) in a ratio to aminopterin of 500:1 is 81% effective in this regard. That CF can substitute for PGA in permitting stilbestrol stimulation of the chick oviduct is further evidence consistent with the theory that CF is a metabolically active form of PGA.

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