# Influence of Sex Hormones on Tolerance to Aminopterin.* (17992)

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(Introduced by A. Mazur.)
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Folic acid as a dietary essential for proper biological activity of estrogen has been demonstrated by the reduction of feathering(1) and oviduct response(2) in stilbestrol-treated chicks, absence of normal estrogen response in immature monkeys(3) and ovariectomized rats(4) on folic acid deficient diets. Impairment of reproductive performance of rats(5) results from a pregestational folic acid deficient diet which is further accentuated by the addition of an antifolic acid chemical. The folic acid antagonist, aminopterin (4-amino-pteroylglutamic acid) used to induce a deficiency, decreases(6) the oviduct response to estrogen in newly metamorphosed frogs and interferes(7) with the depressive influence of estradiol on the rat prostate. A quantitative relationship exists between folic acid and estrogen(2); the frog's oviduct response is increased by folic acid(6). Aminopterin possesses no androgenic activity(7) itself and does not interfere with androgen stimulation. However, a folic acid deficiency produces a more effective comb growth response to androsterone in chicks(4).

Folic acid, therefore, might be instrumental in inhibiting testosterone induced tissue hypertrophy and a deficiency may be the instrument for inhibition of estrogen induced tissue hypertrophy. Since a quantitative relationship between folic acid and estrogen has been established, indicating a higher estrogen response with greater folic acid intake(2), it might be conceivable that the folic acid requirement is increased in estrogen administration. Hertz(2) has pointed out the destruction of bone marrow by prolonged administration of stilbestrol, which might represent an increased folic acid requirement for bone marrow protection. If the sex hormones influence the folic acid requirement of the body, the measurement of tolerance to an induced folic acid deficiency in the presence and absence of these hormones should demonstrate an appreciable difference. Tolerance to aminopterin, a competitive vitamer, serves as a means of quantitatively determining this factor.

*Procedure.* Toxicity studies of aminopterin‡ were conducted on male and female albino (Swiss strain) mice, in 4 groups of experimental animals: (1) mature; (2) mature hormone treated; (3) gonadectomized; (4) immature. Two series were conducted on the mature mice in order to establish the more favorable synthetic folic acid deficient diet for this study. Mature mice in series A were fed, *ad libitum*, the diet 566 of Spicer *et al.* (8) in which intestinal synthesis of folic acid was not inhibited. Mature mice in series B were fed the folic acid deficient diet of Franklin *et al.*(9) in which intestinal synthesis of folic acid was eliminated by the addition of

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1. Oleson, J. J., Hutchings, B. L., Sloane, N. T., *J.B.C.*, 1946, v165, 371.

2. Hertz, R., *Endocr.*, 1945, v37, 1.

3. Hertz, R., PROC. SOC. EXP. BIOL. AND MED., 1944, v67, 113.

4. Hertz, R., Recent Progress in Hormone Research, II, Academic Press, 1948, 161.

5. Nelson, M. M., and Evans, H. M., *J. Nutr.*, 1949, v38, 11.

6. Goldsmith, E. D., Schreiber, S. S., and Nigrelli, R. F., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 299.

7. Brendler, H., *Science*, 1949, v110, 119.

‡ Furnished through the courtesy of Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

8. Spicer, S. S., Daft, F., Sebrell, W. H., and Ashburn, L. L., *U. S. Pub. Health Rep.*, 1942, v57, 1559.

9. Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 368.

TABLE I.
Comparison of Aminopterin Mean Lethal Doses in γ/g in Male and Female Albino Mice.

| Mice | Males | | Females | | Mean diff. | Stand. error diff. | Ratio mean diff. to stand. error diff. |
|---|---|---|---|---|---|---|---|
| | Mean | ± std. dev. | Mean | ± std. dev. | | | |
| Mature A* | 1.62 | .63 | 2.58 | 2.48 | .96 | .17 | 6.75 significant |
| B | 1.31 | .50 | 1.63 | .67 | .36 | .13 | 2.46 " |
| Mature hormone treated† | 1.46 | .18 | 1.81 | .17 | .35 | .15 | 2.38 " |
| Gonadectomized | 1.55 | .24 | 1.58 | .8 | .03 | .16 | .15 not " |
| Immature | 1.54 | .42 | 1.7 | .2 | .16 | .13 | 1.2 " " |

* Series A of mature mice on folic acid deficient diet 566 of Spicer *et al.*(8), intestinal synthesis of folic acid permitted. All other animals were on folic acid deficient diet of Franklin *et al.*(9), intestinal synthesis of folic acid inhibited.

† Males received 5 γ estradiol benzoate injections subcutaneously twice weekly. Females received 50 γ testosterone propionate injections subcutaneously twice weekly.

1% succinyl sulfathiazole to the diet. All animals in other experimental groups received this diet of Franklin *et al.*(9). There were two groups of control animals of both sexes. One group received the folic acid deficient diet(9) and the other a diet made more complete by the addition of folic acid and the exclusion of sulfathiazole(9). The general procedure for all four of the following experimental groups was the same. Mice were weighed at the beginning of the experiment and a periodic checking of weights was made throughout the course of the experiment. Saline solution of aminopterin was administered intraperitoneally daily and the lethal doses were calculated in γ/g on the basis of the total toxic dose and the initial weight.

1. *Mature mice.* Series A consisted of 50 mature males and females which received daily doses of aminopterin varying from .5 to 3γ. Series B consisted of 64 mature males and females which were given doses of 1 to 3γ aminopterin daily.

2. *Mature hormone treated mice.* A group of 64 mature hormone treated males and females were given daily aminopterin doses of 2 and 3γ. Subcutaneous injections of 5γ estradiol benzoate§ to males and 50γ testosterone propionate§ to females were administered twice weekly. This quantity of estradiol is sufficient to produce a full estrogenic smear in ovariectomized females and the quantity of testosterone produces a 100% increase in the size of the seminal vesicle.

3. *Gonadectomized mice.* A group of 54 males and females (2-3 months old) gonadectomized at 3 weeks of age, received daily aminopterin doses of 2 and 3γ.

4. *Immature mice.* Fifty male and female weanling mice received daily aminopterin injections of 3γ.

*Results.* 1. *Mature mice.* In Series A on mature mice, in which intestinal synthesis of folic acid was not inhibited, the range in lethal dose was very wide and varied from .4 to 8.8 γ/g. However, a marked sex difference in tolerance to aminopterin was apparent (Table I) when the mean lethal doses were compared. The difference between the 2.58 for females and the 1.62 for males was very significant and indicated the greater toxicity of aminopterin in males. The intestinal synthesis of folic acid accounted for the large distribution in lethal dose. The ability of some mice to tolerate considerably higher doses was attributed to the variation in the amount of folic acid synthesis. When the mean lethal doses of Series A and B on mature mice were compared, it was demonstrated that inability to synthesize folic acid produced less individual variation as well as lowered tolerance (Table II). The range for Series B was not as great and varied from .5

§ The estradiol benzoate and testosterone propionate were supplied by Ciba Pharmaceutical Products, Inc.

TABLE II.
Comparison of Aminopterin Mean Lethal Doses in γ/g in Mature Albino Mice on Different Diets

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Mature mice compared | Mean diff. | Stand. error diff. | Ratio mean diff. to stand. error diff. | Mean diff. | Stand. error diff. | Ratio mean diff. to stand. error diff. |
| Series A,* B† | .95 | .43 | 2.2 significant | .31 | .18 | 1.78 significant |

* Series A on folic acid deficient diet 566 of Spicer *et al.*(8), intestinal synthesis of folic acid permitted.

† Series B on folic acid deficient diet of Franklin *et al.*(9), intestinal synthesis of folic acid inhibited.

TABLE III.
Comparison of Aminopterin Mean Lethal Doses in γ/g in Various Experimental Groups of Albino Mice on the Same Diet.*

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Mice compared | Mean diff. | Stand. error diff. | Ratio mean diff. to stand. error diff. | Mean diff. | Stand. error diff. | Ratio mean diff. to stand. error diff. |
| Mature (B) with gonadectomized | .24 | .10 | 2.1 significant | .05 | .20 | .26 not significant |
| Mature (B) with immature | .23 | .13 | 1.87 " | .07 | .14 | .48 " " |
| Mature (B) with hormone treated | .15 | .14 | 1.07 not " | .18 | .12 | 1.21 " " |
| Immature with gonadectomized | .01 | .06 | .17 not " | .13 | .14 | .88 " " |

* Folic acid deficient diet of Franklin *et al.*(9), intestinal synthesis of folic acid inhibited.

to 3.1 γ/g. The mean lethal dose of 1.63 for females was significantly higher than the 1.31 for males (Table I). Schoenbach *et al.*(10) observed the same lethal dose in males. A lower tolerance of males to aminopterin was apparent in mice on both diets and the ability to synthesize folic acid increased tolerance of both males and females, though still maintaining a sex difference (Tables I and II).

2. *Mature hormone treated mice.* No influence of testosterone on the tolerance of mature females or estradiol on tolerance of mature males was demonstrated since the same statistically significant sex difference existed (Table I) as shown by a mean difference of .35 γ. In addition there were no significant differences between the mean lethal doses of this group and the untreated mature mice (Table III).

3. *Gonadectomized mice.* Gonadectomy increased the mean lethal dose of males (Table I) to that of normal mature females. The mean lethal doses of gonadectomized males and females were equivalent (Table I) and are 1.55 and 1.58 γ/g respectively. Ovariectomy did not influence the tolerance and was demonstrated by no significant difference in mean lethal doses (Table III) of gonadectomized and normal mature female mice. Castration, however, had a marked effect on the lower male tolerance observed in mature mice and the mean lethal dose was increased by .24 γ (Table III).

4. *Immature mice.* No sex difference in aminopterin tolerance was manifested in immature mice as was similarly demonstrated in

10. Schoenbach, M. D., Goldin, A., Goldberg, B., and Ortega, L. G., *Cancer*, 1949, v2, 57.

gonadectomized mice (Table I). The mean lethal doses of weanlings did not differ significantly from those of gonadectomized mice (Table III).

In all experimental groups, signs of toxicity were apparent. Ruffed hair, bloody diarrhea and weight loss were noticeable. The control animals on folic acid deficient diet long outlived the experimental ones. The average length of survival of mice receiving aminopterin was one month. At the end of 6 months, 6 of the control mice were still living. Two of the males had died after 4 and 5 months.

*Discussion.* The toxicity of aminopterin in mice varies with diet and sex. When mice were capable of intestinal synthesis of folic acid, even as a sole source of supply, the tolerance to aminopterin was considerably increased. Since there was considerable variation in tolerance under these circumstances the mice were fed a more completely folic acid deficient diet by the addition of 1% succinyl sulfathiazole in all subsequent experiments. The sex difference in tolerance observed was attributed to testosterone which must exert a synergistic action with aminopterin. All mice devoid of testosterone (mature, immature and gonadectomized females and immature and gonadectomized males) demonstrated the same tolerance to aminopterin as indicated in similarity of mean lethal dose. Aminopterin was more toxic in mature males since the mean lethal dose was significantly lower than that of the testosterone poor group. Estrogen did not influence the folic acid requirement of the body nor did it exert any effect upon tolerance to aminopterin since its presence in mature females produced no change in mean lethal dose. Though an aminopterin induced folic acid deficiency decreases estrogen response(4,7), the effect of this deficiency must be directly on the end organ.

Testosterone itself is not toxic. However, its presence enhanced the toxic effect of an anti-folic drug such as aminopterin. It is possible that the folic acid requirement of the body was increased in the presence of testosterone. It may be that the mode of action of aminopterin was upon an end organ which was influenced by testosterone and synergism was thereby produced. In mature mice treated with hormones, testosterone was always present with estrogen. However, when administered to females, testosterone did not produce the synergism found in normal mature males even though the quantity would have been enough to increase the seminal vesicle size by 100%. Estrogen, in an amount high enough to produce a full estrogenic smear in gonadectomized females, did not mask the toxic effect of aminopterin in the male and had no influence on whatever end organ aminopterin affected. Mice with and without estrogen tolerated the same lethal dose. For further clarification, gonadectomized mice of both sexes should be given larger doses of testosterone. A specificity of the synergism between testosterone and aminopterin may exist or the action exerts its influence on any induced folic acid deficiency. Other anti-folic drugs should be investigated in the same manner.

*Conclusion.* Folic acid synthesis by intestinal flora decreased the toxicity of aminopterin in mice on an otherwise folic acid deficient diet. The addition of 1% succinyl sulfathiazole to the diet gave more consistent results though aminopterin tolerance was lowered. A lowered tolerance was observed in mature males which is attributed to a synergism between testosterone and aminopterin. A higher mean lethal dose was observed in all mice which did not have testosterone (mature, gonadectomized, immature females and gonadectomized, immature males). Estrogen had no influence on aminopterin tolerance.

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