Effect of Sex Hormones on Conversion of Folic Acid to Citrovorum Factor by Rat.* (21998)

V. M. Doctor and J. B. Trunnell. (Introduced by J. Awapara.)

From Section of Experimental Medicine, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston.

The stimulatory influence of folic acid (PGA) and an inhibitory effect of aminopterin on the tissue growth response to estrogen in the female chick and rat was first reported by Hertz and Tullner(1). Gelfant *et al.*(2) reported that citrovorum factor (CF) partially reverses the inhibitory effect of aminopterin on estrogen induced mitoses in the rat uterus. Aminopterin is reported(3) to inhibit the urinary excretion of CF by rats receiving PGA.

The present report concerns the study of the effect of sex hormones on the *in vivo* conversion of PGA to CF by male rats.

Methods. Adult male rats of the Sprague-Dawley strain were placed individually in metabolism cages equipped with metal collecting funnels. Rats were maintained on Purina laboratory chow (Ralston Purina Co.) starting at the weaning stage and throughout the experimental period. Three separate experiments were conducted. In Exp. 1, 9 rats were randomized into 3 groups of 3 rats each. Group 1 received the Purina laboratory chow unsupplemented. Groups 2 and 3 received a daily supplement of 100 γ PGA by stomach tube. In addition to the latter, Group 3 received intramuscular injections of 7.7 γ estradiol dipropionate in sesame oil. In Exp. 2, 19 rats were randomized into 6 groups, 5 of which consisted of 3 rats each while Group 4 comprised 4 rats. Group 1 received the basal diet unsupplemented. Groups 2, 3 and 4 received a daily supplement of 100 γ PGA by stomach tube. In addition to PGA, Groups 3 and 4 received daily intramuscular injections of 0.5 mg testosterone and 10 γ estradiol dipropionate respectively. Groups 5 and 6 were controls for groups 3 and 4 and received daily intramuscular injections of 0.5 mg testosterone and 10 γ estradiol dipropionate respectively. In Exp. 3, 19 rats were randomized into 5 groups, 4 of which consisted of 4 rats each with the exception of Group 1 which had 3 rats. Group 1 received the basal diet unsupplemented. Groups 2, 3, 4 and 5 received a daily supplement of 100 γ PGA by stomach tube. In addition to PGA, Group 3 received daily intramuscular injections of 0.5 mg testosterone, Group 4 received daily intramuscular injections of 10 γ estradiol dipropionate, while Group 5 received daily injections of same amounts of both the sex hormones. Urine collections were started 24 hours after the rats had been maintained on the respective diets and were continued daily for a one to 2-week test period. The 24-hour urine collections from the rats of the same group were combined and an aliquot of the latter was filtered, neutralized and autoclaved for 30 minutes at 120°C. The heated samples were cooled, reneutralized, diluted and assayed. The CF content of the urine samples was determined by using the Bacto-Cf Assay Medium (Difco Laboratories). *Leuconostoc citrovorum* ATCC 8081† was used as the test organism, and Leucovorin (Lederle) was employed as the standard. The cultures were incubated 48 hours at 37°C and growth was measured by turbidimetric procedure. Since leucovorin has been reported to be half as active as the CF isolated from a horse liver(4), the microbiological assay values were divided by 2 in order to express the results in terms of

* This work was supported in part by a grant from the American Cancer Society. Leucovorin (Lederle) and folic acid were generously supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y. The authors are indebted to Guy R. Fain and E. M. Cooney for technical assistance.

† A recent report (Felton, E. A., and Niven, C. F., *J. Bact.*, 1953, v65, 482) concerning a taxonomic study on the culture *Leuconostoc citrovorum* ATCC 8081 suggests that the latter organism is a typical strain of *Pediococcus cerevisiae* as described earlier by Pederson (*Bact. Rev.*, 1949, v13, 225).

TABLE I. Effect of Sex Hormones on Urinary Excretion of Citrovorum Factor by Rats Receiving PGA.

Exp.	No. of rats	Supplement* to basal/day	Urinary excretion of CF, mγ/rat/day 1st wk	2nd wk
1	3	0	135	
	3	PGA, 100 γ	750	
	3	*Idem* + EDP,† 7.7 γ	1125	
2	3	0	155	
	3	PGA, 100 γ	785	
	3	*Idem* + Test.,† .5 mg	810	
	4	" + EDP, 10 γ	1320	
	3	Test., .5 mg	75	
	3	EDP, 10 γ	230	
3	3	0	232	170
	4	PGA, 100 γ	902	1068
	4	*Idem* + Test., .5 mg	1015	960
	4	" + EDP, 10 γ	1320	1454
	4	" + Test., .5 mg + EDP, 10 γ	1518	1495

* PGA administered by stomach tube while sex hormones were given by intramuscular injections.
† EDP = Estradiol dipropionate in sesame oil. Test. = Aqueous suspension of testosterone.

the naturally occurring CF. At the end of the test period the rats from Exp. 2 were sacrificed, accessory sex organs and the testis removed, blotted and weighed.

Results. Administration of 100 γ PGA to male rats receiving a practical diet increases 4 to 6-fold the urinary excretion of CF (Table I). This increase is enhanced 50% by daily administration of 7.7 γ or 10 γ of estradiol dipropionate. On the other hand daily administration of 0.5 mg testosterone to male rats receiving PGA does not increase the urinary excretion of CF. The stimulatory effect of estradiol dipropionate on the CF excretion is not reversed by simultaneous administration of testosterone. It is interesting to note that rats receiving estradiol dipropionate alone also show a 50% increase in CF excretion, while the rats receiving testosterone alone show a 50% decrease in CF excretion.

It is apparent from the results in Table II that administration of 100 γ of PGA to the male rats does not influence the weight of the accessory sex organs or the testis. Estradiol dipropionate alone or together with PGA markedly decreases the size of the accessory sex organs and the testis. On the other hand testosterone alone or in combination with PGA significantly increases the size of the accessory sex organs.

Discussion. The results presented indicate clearly that *in vivo* conversion of PGA to CF, as measured by the urinary excretion of CF after administration of PGA is enhanced by estradiol dipropionate but is not influenced by administration of testosterone. It is interesting to note that the stimulatory effect of estradiol dipropionate on CF excretion is not reversed by the simultaneous administration of testosterone. Administration of 10 γ estradiol dipropionate alone gives a 50% increase in CF excretion, while the latter decreases by 50% on administration of 0.5 mg testosterone. Estradiol dipropionate markedly decreases while testosterone increases the size of the accessory sex organs. Under these conditions supplementing of PGA does not influence the size of the accessory sex organs. The administration of ascorbic acid to rats receiving PGA has been reported to enhance the urinary excretion of CF(3). Dietary homocysteine and cysteine enhance the urinary excretion of CF by rats receiving PGA

TABLE II. Effect of PGA and Sex Hormones on the Accessory Sex Organs and Testis in the Male Rat.

Supplement* to basal/day	Final body wt, g	Accessory sex organs† Seminal vesicle	Coagulating gland	Ventral prostate	Dorsal prostate	Testis
		mg/100 g				
0	342	108	36	131	65	444
PGA, 100 γ	361	100	45	150	68	453
Idem + Test., .5 mg	352	173	99	236	135	452
" + EDP, 10 γ	312	45	12	23	21	357
Test., .5 mg	354	176	75	262	153	453
EDP, 10 γ	301	51	14	21	12	320

* PGA administered by stomach tube while sex hormones were given by intramuscular inj.
† The figures denote mg of organs/100 g body wt.

(5). Extensive studies on the effects of dietary factors on the CF excretion have not been reported. A very interesting aspect of folic acid deficiency in the rat is the diminished response to estradiol induced uterine growth(1). An interference with metabolism of C^{14} labeled estrone in aminopterin-treated rats is reported by Trunnell *et al.*(6).

Summary. Administration of estradiol dipropionate alone or together with PGA enhances the urinary excretion of CF by male rats. Testosterone alone gives a 50% decrease in the CF excretion but is without significant effect when given in combination with PGA. The stimulatory effect of estradiol dipropionate can not be reversed by testosterone in the amounts tested.

1. Hertz, R., and Tullner, W. W., *Ann. N. Y. Acad. Sci.*, 1950, v52, 1260.

2. Gelfant, S., Meyer, R. K., and Ris, H., *J. Exp. Zool.*, 1955, v128, 219.

3. Welch, A. D., Nichol, C. A., Anker, R. M., and Boehne, J. W., *J. Pharmacol. Exp. Therap.*, 1951, v103, 403.

4. Keresztesy, J. C., and Silverman, M., *J. Am. Chem. Soc.*, 1951, v73, 5510.

5. Doctor, V. M., and Trunnell, J. B., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 498.

6. Trunnell, J. B., Bowman, W. E., and Hildebrandt, H. E., Abstract, *J. Clin. Endocrinol. and Metab.*, 1954, v14, 796.

Received September 7, 1955. P.S.E.B.M., 1955, v90.

Mechanism of Protective Effect of Hydrocortisone in Staphylococci Infected Adrenalectomized Mice.*† (21999)

R. D. HIGGINBOTHAM AND THOMAS F. DOUGHERTY.

From the Department of Anatomy, University of Utah College of Medicine, Salt Lake City.

The reduced natural resistance of Addisonian patients as well as of adrenalectomized animals is a general phenomenon not specific for any one type of infection or intoxication. Although the mechanism by which adrenalectomy influences resistance remains relatively obscure, replacement therapy with adrenal cortical hormones has been shown to be beneficial(1,2). On the other hand, adrenal hormones may be detrimental to host resistance(3). It has been suggested, however, that the beneficial effects of cortisone therapy are to be derived only from dosages based on requirement(4,5). In this regard, Robinson, *et al.*(6), have recently pointed out that the detrimental effect of cortisone in resistance to pneumococcal infection in rats is primarily due to overdosage with the hormone, and that an optimal dosage schedule of cortisone can significantly enhance the host resistance of either adrenalectomized or intact infected animals. Hill, *et al.*(7), have described similar findings with systemic moniliasis in adrenalectomized mice treated with cortisone. Germuth, *et al.*(8), found that cortisone-treated rabbits could successfully resist an intravenous inoculum of *Staphylococcus aureus* which was fatal to approximately 50% of the untreated controls. They noted that the bacteria rapidly disappeared from the blood after injection in either treated or untreated animals. Kleiger and Blair(9) have described the acute death of experimental animals resulting from an inoculation of toxigenic staphylococci as an *in vivo* production of toxin by the bacteria.

It has been observed in this laboratory, that a strain of staphylococci which regularly produced a rapidly fatal bacteremia in mice after intraperitoneal inoculation proved to be relatively innocuous if given by the intravenous route. However, adrenalectomy reduced resistance to an intravenous challenge with this microorganism by a factor of at

* Work supported by Grant No. DA-49-007-MD-130, Department of the Army.

† The authors wish to acknowledge the technical assistance of Miss Martha Chamberlin in this study, and their indebtedness to Dr. Paul S. Nicholes for the use of the high frequency oscillator.