Response of Vitamin B_6-Deficient Rats to Non-Hypophyseal Gonadotrophins.* (26679)

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Previous studies have shown the consistent occurrence of reproductive disturbance in adult female vitamin B_6-deficient rats(1,2). Reproductive failure was apparently due to lack of ovarian hormones as pregnancy could be maintained in 90% of such rats by daily injection of estrone and progesterone(3) at dosage levels known to be effective in the hypophysectomized-oophorectomized rat(4). Injection of pituitary gonadotrophins was, however, less successful in maintaining pregnancy in B_6-deficient rats, a finding which suggests the possibility of a decreased ovarian response to these hormones(5). Ovarian response in virgin B_6-deficient rats to injected hypophyseal follicle-stimulating hormone (FSH) was markedly decreased when compared with the response obtained in adult hypophysectomized rats, whereas only a slightly decreased response to hypophyseal interstitial-cell-stimulating hormone (ICSH) was observed in B_6-deficient rats(6). The present report‡ describes the response of virgin B_6-deficient rats as compared with normal immature rats to graded levels of 2 non-hypophyseal gonadotrophins, human chorionic gonadotrophin (HCG) and equine gonadotrophin (PMS).

Methods. Normal female rats (Long-Evans strain) were given the Vit. B_6-deficient diet§ when 80 to 85 days of age. Vaginal smears were examined daily and the rats weighed and their condition described every 5 days. After 60 to 65 days on the diet, those B_6-deficient animals which showed definite signs of deficiency, *e.g.*, cessation of growth and progressive acrodynia, were distributed into equivalent groups on the basis of body weight and length of anestrus. The rats had been anestrous for an average of 44 days and had lost approximately 10% of their body weight. Varying levels of HCG‖ were injected subcutaneously once daily for 4 successive days; PMS‖ was given in a single subcutaneous dose. Normal immature rats, 24 days of age and maintained on a stock diet of natural foodstuffs,¶ were injected with the same levels of these hormones as B_6-deficient animals. All dosage levels were repeated at least once for both types of animals. Normal immature rather than hypophysectomized rats served as controls in this study, since hypophysectomized rats are less responsive to low levels of HCG and PMS(9). All rats were autopsied 96 hours

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§ The vit. B_6-deficient diet(3,5,6) consisted of 24% alcohol extracted casein, 64% sucrose, 8% hydrogenated cottonseed oil (Crisco or Primax), 4% salts No. 4 and 0.5 mg% deoxypyridoxine, the vit. B_6 antimetabolite. Crystalline vitamins per kilo of diet were 300 μg *d*-biotin, 5 mg 2-methyl-1, 4-naphthoquinone, 5 mg thiamine HCl, 5.5 mg pteroylglutamic acid, 10 mg riboflavin, 10 mg p-aminobenzoic acid, 20 mg niacin, 50 mg *d*-calcium pantothenate, 400 mg inositol, and 1.0 g choline chloride. Each rat received weekly a fat-soluble vitamin mixture containing 800 USP units vit A, 115 chick units vit D, 6 mg synthetic *dl*-alphatocopherol, and 650 mg corn oil (Mazola).

‖ HCG ("Antophysin") was prepared by Winthrop Laboratories and PMS ("Gonadin") by Cutter Laboratories.

¶ Composition of this stock diet (XIV) is given in Srebnik *et al.*(8).

TABLE I. Response to HCG in Vit. B_6-Deficient and in Normal Immature Rats.

Total dose, IU	No. of rats	Ovaries			Uterine wt, mg
		Wt, mg	Follicles*	Interstitial cells	
			Vit. B_6-deficient		
0	22	34 ± 2†	sm, m	Atrophic	109 ± 5
1.25	6	31 ± 3	" "	"	117 ± 8
2.5	8	28 ± 9	sm, m, few ml-l	"	189 ± 21
5.0	8	48 ± 5	m-l, CL	Repair	240 ± 37
			Normal immature		
0	10	19 ± 1	sm, few m	Epithelioid	22 ± 1
1.25	10	18 ± 2	" "	"	23 ± 2
2.5	10	18 ± 1	sm, m, ml-l	"	116 ± 18
5.0	5	28 ± 3	few ml-l, CL	"	102 ± 6

* Follicles were measured with an ocular micrometer and designated as small (s) = 375 μ, small-medium (sm) = 450-500 μ, medium (m) = 550-600 μ, medium-large (ml) = 700-750 μ, and large (l) = 800-1000 μ. The presence of new corpora lutea (CL) was recorded.

† Stand. error of mean.

after first injection. Ovaries and uteri were weighed at autopsy. The ovaries were fixed in Bouin's fluid, embedded in nitrocellulose, serially sectioned, and stained with hematoxylin and eosin. Criteria for ovarian responses were those used in previous studies (6,8). Follicle-stimulating activity was detected by an increase in size and number of ovarian follicles as compared with those in uninjected controls, and interstitial-cell-stimulating activity by a histologically detectable response designated as partial repair of the atrophic interstitial cells(10). Additional criteria were corpus luteum formation, significant increase in uterine weights, estrous vaginal smears in adult animals and vaginal openings in immature rats.

Results. The degree of follicular development was approximately the same in the ovaries of B_6-deficient and normal immature rats before hormone injections were started; both contained small-medium and a few medium-sized follicles. In uninjected B_6-deficient animals the ovarian interstitial cells were atrophic with pyknotic nuclei and degenerating, pale-staining cytoplasm; residual corpora lutea similar to those observed after hypophysectomy were present. No corpora lutea were present in the ovaries of normal immature rats and the interstitial tissue was epithelioid.

Vit. B_6-deficient rats responded to the same dosage levels of HCG as normal immature rats; follicular maturation and increased uterine weights occurred in both groups when 2.5 IU were given (Table I). Injection of 5 IU caused corpus luteum formation in both groups and produced vaginal opening in immature rats and estrous vaginal smears in B_6-deficient animals. In addition, the atrophic interstitial tissue was stimulated in B_6-deficient rats with this dosage level.

Vit. B_6-deficient rats were somewhat less sensitive to PMS than normal immature rats (Table II). Follicular development and increases in uterine weight were observed in some normal immature rats when 0.5 IU were given whereas B_6-deficient animals required one IU for this effect. Corpus luteum formation occurred in all normal immature rats injected with one IU but B_6-deficient rats needed 2 IU for this response. Partial repair of interstitial tissue and vaginal estrus were also observed in B_6-deficient animals given 2 IU. Higher dosage levels (5-10 IU) significantly increased the number of follicles and corpora lutea in normal immature rats but not in B_6-deficient animals.

Discussion. This study has shown that Vit. B_6-deficient rats respond to low levels of the non-hypophyseal gonadotrophins, although they required slightly higher dosages of PMS than normal immature rats for equivalent ovarian effects. These low levels of HCG and PMS which cause stimulation of follicular growth in B_6-deficient rats may be contrasted with the high levels of exogenous hypophyseal FSH required for the same effect. Wooten *et al.*(6) have shown that 16

TABLE II. Response to PMS in Vit. B_6-Deficient and in Normal Immature Rats.

Total dose, IU	No. of rats	Ovaries			Uterine wt, mg
		Wt, mg	Follicles	Interstitial cells	
			Vit. B_6-deficient		
0	24	34 ± 2	sm, m	Atrophic	105 ± 4
.5	8	33 ± 4	" "	"	120 ± 14
1	12	32 ± 3	m, few ml-l	"	187 ± 22
2	9	43 ± 2	", few ml-l, CL	Partial repair	235 ± 19
5	8	50 ± 5	" " " "	" "	221 ± 19
10	8	52 ± 3	" " " "	Repair	196 ± 15
			Normal immature		
0	19	17 ± 1	sm, few m	Epithelioid	27 ± 2
.5	14	16 ± 1	m, few ml-l	"	63 ± 12
1	15	25 ± 1	few m-ml, CL	"	110 ± 3
2	13	26 ± 1	" "	"	81 ± 8
5	9	39 ± 7	many m-ml, CL	"	73 ± 7
10	9	56 ± 11	" "	"	84 ± 12

Abbreviations as in Table I.

RU of injected hypophyseal FSH were needed for follicular stimulation in B_6-deficient rats as compared with 2 RU for normal immature or adult hypophysectomized animals. It is generally assumed that the follicle-stimulating effect of low levels of HCG and PMS is due to release of FSH from the animal's own pituitary, the hormone then synergizing with the ICSH-like activity of the injected non-pituitary gonadotrophins (9). Since the pituitaries of B_6-deficient rats contain considerable FSH(11) this may have been released following injection of HCG and PMS. This study suggests that B_6-deficiency does not affect the ovarian response to endogenous FSH and that the observed insensitivity in this deficiency to exogenous hypophyseal FSH preparations may be of different origin.

The only comparable study of response to these gonadotrophins(8) has shown that rats deprived of dietary protein for one month responded to lower levels of HCG and PMS than did normal immature rats. The factors responsible for the greater effectiveness of these non-hypophyseal gonadotrophins in protein-deficient rats are apparently not operative in B_6-deficient animals.

Summary. The response of Vit. B_6-deficient rats to graded doses of 2 non-hypophyseal gonadotrophins, HCG and PMS, was determined and compared with that of normal immature rats. Follicular growth, corpus luteum formation, and increased uterine weights occurred with the same levels of HCG in both B_6-deficient and normal immature rats whereas slightly more PMS was required by the B_6-deficient than the normal immature rat to obtain an equivalent ovarian response.

1. Nelson, M. M., Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 274.

2. ———, *J. Nutrition,* 1951, v43, 281.

3. Nelson, M. M., Lyons, W. R., Evans, H. M., *Endocrinol.,* 1951, v48, 726.

4. Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 65.

5. Nelson, M. M., Lyons, W. R., Evans, H. M., *Endocrinol.,* 1953, v52, 585.

6. Wooten, E., Nelson, M. M., Simpson, M. E., Evans, H. M., *ibid.,* 1958, v63, 860.

7. Nelson, M. M., Wooten, E., Evans, H. M., *Fed. Proc.,* 1957, v16, 394.

8. Srebnik, H. H., Nelson, M. M., Simpson, M. E., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 57.

9. Simpson, M. E., in Cole, H. H., Cupps, P. T., ed., *Reproduction in Domestic Animals;* 59-110, N. Y. and London, 1959.

10. Evans, H. M., Simpson, M. E., Tolksdorf, S., Jensen, H., *Endocrinol.,* 1939, v25, 529.

11. Wooten, E., Nelson, M. M., Simpson, M. E., Evans, H. M., *ibid.,* 1955, v56, 59.

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