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Effect of Vitamin-E Deficiency on Amount of Gonadotrophin in the Anterior Pituitary of Rats.* (17486)

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Associated with a deficiency of vitamin E in males there are degenerative changes in the germinal epithelium and disappearance of spermatogenesis without apparent morphological alteration of the interstitial cells. The failure of vitamin-E deficient female rats to become pregnant is apparently due to a disturbance of the implantation process rather than the absence of ovulation; there is no direct proof of ovarian dysfunction.(1,2) Since normal function of the ovaries and testes is dependent upon the anterior pituitary, several authors have compared the morphology or the gonadotrophic potency of the anterior pituitary of normal rats and of rats on a diet deficient in vitamin E. In males, van Wagenen(3) found castration changes in the basophils of

* Aided by grants from E. R. Squibb and Sons, the United States Public Health Service, and the Williams-Waterman Fund of the Research Corporation.

1. Kaunitz, H., and Slanetz, C. A., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 334.

2. Blandau, R. J., Kaunitz, H., and Slanetz, C. A., *J. Nutr.*, 1949, v38, 97.

3. Van Wagenen, G., *Anat. Rec.*, 1925, v29, 298.

the gland. Her results were extended and confirmed by Koneff.(4) The anterior pituitary of female E-deficient rats likewise showed the morphological changes characteristic of spaying, according to Joel.(5) Both Koneff and Joel also described a hypertrophy and a decrease in the number of oxyphils. In conformity with these findings, Nelson(6) reported that the increase in the gonadotrophic potency of the pituitary of E-deficient males approached that of the gland of castrates. He detected no difference in the amount of gonadotrophin in the pituitaries of normal and E-deficient females. However, McQueen-Williams(7) concluded that glands from E-deficient female rats contain about twice as much gonadotrophin as those from normal rats or E-deficient rats receiving the vitamin for 2 weeks or longer. In contrast with the foregoing reports, Biddulph and Meyer(8) stated that pituitary powder from vitamin-E deficient male or female rats produced the same weight change in the ovaries of hypophysectomized rats used for assay as normal pituitary powder from a corresponding sex. However, they believed that male pituitaries from E-deficient rats contained an increased amount of luteinizing (interstitial-cell stimulating) hormone. The gonadotrophin in all these experiments was assayed in immature female rats whereas Rowlands and Singer(9) employed a different method: the production of ovulation in estrous rabbits. According to their findings, the pituitary of the E-deficient female rat contained less gonadotrophin than the normal although this may have depended upon a reduction of a gonadotrophin not detectable in the assays in rats.

With the exception of the experiments of Biddulph and Meyer, all the assays in the work cited above were performed in animals with intact pituitaries. It seemed of interest to reinvestigate the problem by performing the assays in hypophysectomized male rats so that (a) any contribution of the anterior pituitary of the assay animals to the gonadotrophic effect of the glands of donors could be excluded, and, (b), interstitial-cell stimulation could be better evaluated from effects on the anterior prostate. The female donor groups were of special interest since studies(2) of their ovarian function had already been completed.

TABLE I.
Composition of Vit. E Deficient Diet.

Basal mixture	%
Casein, crude	30
Cerelose	54
Lard, commercial	10
Salt mixture (Hawk-Oser)	4
Celluration	2
Supplements of basal mixture	**mg/kilo**
Thiamine chloride	2
Riboflavin	4
Pyridoxine	4
Calcium pantothenate	10
p-Amino benzoic acid	300
Choline	1000
Inositol	1000
Vit. K	4
Oleum percomorphum	200

Methods. Albino rats of a highly inbred stock were maintained on the vitamin-E deficient diet given in Table I from birth until sacrificed. The control rats received the same diet supplemented by 3 mg of synthetic dl-alpha-tocopherol acetate† per 100 g of the diet, permitting a daily intake of 300 μg in contrast to 30 micrograms of the vitamin afforded by the deficient diet. At average ages ranging from 315 to 469 days, corresponding groups of vitamin-E deficient and control rats were sacrificed, and the anterior pituitary gland from each rat was removed, carefully dissected and weighed. The pituitaries from each group of rats were triturated together in a mortar into a homogenous fine suspension in distilled water, transferred to an ampoule, quickly frozen and dried in a frozen state under high vacuum.

For the bioassay of its gonadotrophic po-

4. Koneff, A. A., *Anat. Rec.*, 1939, v74, 383.

5. Joel, C. A., *Monatschr. Geburtsh. Gynak.*, 1943, v116, 288.

6. Nelson, W. O., *Anat. Rec.*, 1931, v56, 241.

7. McQueen-Williams, M., *Anat. Rec.*, 1934, v58 (Suppl.), 77.

8. Biddulph, C., and Meyer, R. K., *Amer. J. Physiol.*, 1941, v132, 259.

9. Rowlands, I. W., and Singer, E., *J. Physiol.*, 1936, v86, 323.

† Synthetic dl-alpha-tocopherol acetate was kindly supplied by Dr. Leo Pirk of Hoffmann-La Roche.

TABLE II.
Gonadotrophic Potency of the Anterior Pituitary of Normal and Vitamin-E Deficient Rats.

Group	Sex	Donor rats: Avg age, days	Donor rats: No.	Donor rats: Body wt,* g	Wt of ant. pit.: Fresh,* mg/100 g	Wt of ant. pit.: Dry, mg/100 g
Normal	M	325	12	375 ± 13.4	2.29 ± 0.11	.41
E-deficient	M	315	13	255 ± 12.3	2.86 ± 0.24	.54
Normal	F	388	12	254 ± 8.7	5.11 ± 0.04	.79
E-deficient	F	469	12	237 ± 11.5	4.35 ± 0.20	.71

Hypophysectomized male recipient rats†

No.	Ant. pit. powder: Source	Dose, mg	Wt of testes,* mg	P‡	Wt of sem. ves.,* mg	P‡	Wt of ant. prost.,* mg	P‡
23			121.3 ± 3.40		4.68 ± .33		10.91 ± .51	
27	Normal ♂	.1	154.2 ± 3.39	<.01	5.90 ± .36	>.6	17.07 ± .71	.1
29	E-defic. ♂	.1	170.1 ± 1.28		6.14 ± .34		19.05 ± .90	
29	Normal ♀	.2	147.2 ± 3.71	<.02	4.99 ± .23	>.5	16.88 ± .76	>.2
29	E-defic. ♀	.2	162.6 ± 5.10		5.19 ± .25		17.92 ± .73	

* Mean and standard error of the mean.
† Average wt range of experimental groups: 40.2 to 41.0 g; average wt of controls: 35.9 g.
‡ Comparison of means calculated according to "t" method of Fisher.

tency, a fine suspension of the dried powder in physiological saline was made so that each 2 cc contained the total amount of the gland to be injected into one rat. The total dose for groups of rats for each day was placed in separate vials which were kept frozen until injected. One-half cc of the suspension was given subcutaneously on each of the 4 consecutive days, starting one day after operation, to a male weanling rat hypophysectomized at 21 days of age by the parapharyngeal approach.(10) The rats were sacrificed 24 hours after the last injection; the testes, seminal vesicles and anterior prostates were carefully removed, freed of connective tissue and weighed on a sensitive torsion balance. Any rats with remnants of the pituitary as detected by examination of the sella with a binocular microscope were excluded. A considerable number of hypophysectomized rats was sacrificed prior to the experiments to determine a suitable dose of pituitary powder necessary to initiate hypertrophy of the sex glands. It was found to be 100 μg of the male and 200 μg of the female dried pituitary powder.

Results. It will be seen from Table II that the weight of the testes of the immature hypophysectomized rats which received injections of anterior pituitary glands from either male or female vitamin-E deficient rats was greater than that of rats receiving corresponding doses of the gland from normal male or female rats. The weights of the seminal vesicles and anterior postrates of the rats which received anterior pituitary from normal or vitamin-E deficient male and female donors showed, however, no significant difference.

Discussion. It is evident from the foregoing results that there was a significant increase in the gonadotrophic potency of the anterior pituitary glands of vitamin-E deficient rats of both sexes. These findings, however, did not indicate whether the increased gonadotrophin content of the anterior pituitary of the vitamin-E deficient rats was owing to an increased production or to a decreased secretion or to both.

The fact that only the weights of the testes and not those of the anterior prostates or seminal vesicles of the hypophysectomized rats were greater in rats which received injections of anterior pituitary from vitamin E-deficient

10. Smith, P. E., *Am. J. Anat.*, 1930, v45, 205.

rats might be attributed to an increase in the pituitary follicular stimulating hormone (FSH) without concomitant increase in the interstitial-cell stimulating hormone (ICSH). Such an interpretation would be in conformity with the well known fact that vitamin-E deficiency causes degenerative changes of the germinal epithelium without evident histological or functional changes in the interstitial cells in the testes. It is possible that differences in the quantities of the two gonadotrophins could be revealed if several different dose levels were to be used for the comparisons. However, no conclusion can be drawn from the experiments reported in Table II.

That the anterior pituitary of the vitamin-E deficient female rats also contained an increased amount of gonadotrophic hormone is of interest because of the controversy concerning ovarian function in vitamin-E deficiency. Although the ovaries of old, E-deficient rats showed a greater accumulation of connective tissue and pigmentation than those of controls, observations of ovarian function, such as the production of ova followed by fertilization, revealed no abnormalities. It remains to be investigated, however, whether the increased gonadotrophin content of the pituitary gland indicates a disturbance of ovarian function or whether a different explanation must be sought.

Summary. The gonadotrophin content of the anterior pituitary taken from vitamin-E deficient rats, 315 to 469 days of age, was determined. The weights of testes, seminal vesicles and anterior prostates of groups of male rats hypophysectomized at 21 days of age and subsequently injected with suspensions of frozen-dried anterior pituitaries were compared. The testes of the hypophysectomized rats which received the pituitary suspension from vitamin-E deficient male or female rats were significantly heavier than those of rats which received pituitary powder from the controls. There was no difference in the weights of the seminal vesicles or anterior prostates.

It is concluded that the gonadotrophin content of the anterior pituitary gland is elevated in older vitamin-E deficient male and female rats. The relationship of these findings to the changes in the gonads in vitamin-E deficiency are discussed.

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A Probable Acid Mucopolysaccharide Present in Granulation Tissue.* (17487)

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(Introduced by M. Heidelberger)

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This paper is a preliminary report of observations made on the healing of experimental fractures in the rabbit. Certain histological and chemical reactions of the reparative tissue have led us to the belief that there is present in the early granulation tissue of bone repair, an acid mucopolysaccharide which may play a role in tissue healing. Tissue sections of rabbit callus from one to 10 days after fracture were stained metachromatically with a 1% solution of toluidine blue. Early specimens take up only a small quantity of the dye. As the granulation tissue forms, however, an increasing metachromasia appears, and as the fibrous connective tissue becomes more abundant and differentiated, the ability to stain metachromatically with toluidine blue diminishes. Lison(1) attributed this ability to stain metachromatically to the

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1. Lison, L., *Soc. de Biol. Compt. Rend.*, 1935, v118.