

Influence of Alpha Tocopherol on Implantation in Old Rats.*

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Investigations concerned with the fertility of female rats on vitamin E deficient diets have been mainly focused on the "resorption gestation." The question of implantation in vitamin E deficiency has received much less attention.¹

In these experiments, the rate of implantation of female rats at different age periods on a vitamin E deficient diet was compared with that of rats on the same diet completed by addition of alpha tocopherol.

Methods. 1130 mating experiments were carried out on 293 albino rats of a highly inbred colony. The ancestry of these rats had been kept for 7 or 8 generations on a modified "Evans-Burr" diet containing 20% commercial lard. When these females gave birth to a litter, they were immediately placed on a simplified diet on which they, their offspring and subsequent generations were kept without interruption.

The diet consisted of:

	parts
commercial lard	10
crude casein	30
cerelease	54
celluration	2
salt mixture	4
	mg/kilo
pyridoxine	4
thiamine chloride	2
riboflavin	4
choline	1000
vitamin K	4
p-aminobenzoic acid	300
calcium pantothenate†	10
oleum percomorphum	200

Its tocopherol content was repeatedly checked according to the method of Kaunitz-Beaver² and was found to be below .4 mg per 100 g of diet. From these determinations and from those of Quaife and Harris,³ it can be estimated that the daily intake of the adult rats was in the neighborhood of 20-40 micrograms of tocopherols, because lard was the only measurable source of tocopherol in the diet. The latter was freshly prepared at least once weekly and kept under refrigeration. Five generations of rats raised on this diet were used for the tests. The diet had previously proved to be satisfactory in studies of growth and bioassays (Kaunitz;⁴ Kaunitz and Beaver⁵).

Controls derived from the same colony were kept on the same diet augmented by 4 mg synthetic dl alpha tocopherol acetate‡ per 100 g diet. The weights of these animals, charted according to Zucker and Zucker,⁶ were satisfactory. Females of the first through fifth generation were used approximately in the same proportion as those on the deficient diet.

Mating was carried out by leaving the female for 5 days with the selected male. On and subsequent to the 15th day after mating had begun, the females were examined for the "implantation sign" and their weight was taken on the same scale daily except for occasional omissions. If the implantation sign

² Kaunitz and Beaver, *J. Biol. Chem.*, 1944, **156**, 653.

³ Quaife and Harris, *Ind. and Eng. Chem. (Anal. Edit.)*, 1946, **18**, 707.

⁴ Kaunitz, *J. Nutrition*, 1946, **32**, 327.

⁵ Kaunitz and Beaver, *J. Biol. Chem.*, 1946, **166**, 205.

‡ Dr. Leo Pirk of Hoffmann-La Roche very kindly supplied us with alpha-tocopherol and part of the synthetic vitamins.

⁶ Zucker and Zucker, *J. Gen. Phys.*, 1942, **25**, 445.

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¹ Emerson and Evans, *J. Nutrition*, 1939, **18**, 501.

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TABLE I.
Mating Experiments on Rats on a Purified Diet Containing About 100-400 Micrograms Tocopherols per 100 g, According to Age and Tocopherol Supplement.

Alpha tocopherol acetate supplement	No. of experiments	No. of pregnancies	% Implantations	No. of Litters	No. of Resorptions	Litters in % of pregnancies
Age 53-150 Days.						
None	47	31	66	3	28	10
Single dose of .5-1 mg	48	36	75	9	27	25
" " " 1.1-2 "	51	40	78	23	17	58
" " " 2.1-3 "	11	9	82	8	1	89
4 mg per 100 g diet	55	48	87	48	0	100
Age 151-240 Days.						
None	53	29	56	4	25	14
Single dose of .5-1 mg	37	27	73	9	18	33
" " " 1.1-2 "	47	30	64	20	10	67
" " " 2.1-4 "	18	15	83	14	1	93
4 mg per 100 g diet	22	16	73	15	1	93
Age 241-360 Days						
None	22	4	18	2	2	?
Single dose of .5- 2 mg	24	16	66	4	12	25
" " " 2.1- 4 "	14	7	50	4	3	57
" " " 4.1-10 "	26	15	58	8	7	53
4 mg per 100 g diet	32	24	75	24	0	100
Age 361 Days and Over.						
Single dose of 0 - 3 mg	20	2	10	0	2	0
" " " 3.1-10 "	29	12	41	2	10	18
"Massive" doses	50	11	22	9	2	82
4 mg per 100 g diet	33	20	61	17	3	85

was followed by weight gain and thereafter by gradual weight loss, a resorption gestation was recorded. In 22 not entirely clear cut cases, autopsies were performed. A litter was registered regardless of the number of dead and living young. Animals which persistently failed to become pregnant were excluded from the charts in accordance with the practice of Evans and Burr.⁷ Only females of 150 g or over were used for breeding.

With this technique, pregnancy was observed in 36 out of 43 mating tests (84%) in rats on "Evans-Burr" diet, and in 87% of the younger rats on the complete diet. These data are in good agreement with Evans' and Burr's⁷ observation on the implantation rate in a rat colony kept on a complete diet, and with those reported by Goettsch and Pappenheimer.⁸

In the earlier experiments, males of proven fertility kept on Rockland rat diet were used. In over 900 of the experiments, however, the males raised on the complete simplified diet were mated. In case no pregnancy was ob-

served, the result was charted only if the male was fertile before and after the negative test.

The females were used repeatedly. On the 5th day after the mating had commenced and after the male had been removed, they were either left on the diet or they were given single supplements of alpha tocopherol acetate diluted with 30 g of the experimental diet. The cages were cleaned before the supplements were offered, and no additional food was given until the supplement was completely consumed, usually within 3 days. The single doses amounted to .5-3 mg alpha tocopherol during the first 5 months of life, to .5-4 mg from the fifth to the eighth months, and thereafter to .5-10 mg.

Fifteen female rats kept on the deficient ration, were given 1-2 drops (30-60 mg) of alpha tocopherol acetate at intervals of 1-2 weeks, after they had reached the age of one year.

After a resorption gestation or after the weaning of a litter (towards the end of the fourth week), at least 4 weeks elapsed before the female was mated again.

It is probable that some pseudopregnancies were recorded as resorptions, because the

⁷ Evans and Burr, *The Antisterility Vitamine Fat Soluble E*, Univ. Cal. Press, Berkeley, 1927.

⁸ Goettsch and Pappenheimer, *J. Nutrition*, 1941, **22**, 463.

nature of these experiments excluded verification by autopsy in the majority of the cases. A further source of error was the repeated administration of tocopherol to the same rat. Both these sources of error should reduce the number of rats in which failure of implantation was recorded. The statistical differences between the groups thus became even more significant.

Experiments. In Table I are computed the results of the mating tests in relation to age and tocopherol supplement. After the 240th day there is an abrupt reduction in the percentage of implanting in the animals without tocopherol supplements which is in agreement with Emerson's and Evans' observations. Single supplements of .5-2 mg after the eighth, but before the twelfth months, and doses of 3-10 mg after one year increased the rate of implantation significantly. The "mean fertility dose" of the animals of 8-12 months was roughly 3-4 mg, and after one year litters were observed only twice with single doses up to 10 mg.

When "massive doses" of tocopherol (30-60 mg in intervals of 1-2 weeks in addition to the "complete" diet) were administered to rats after at least one year on the deficient diet, about one-half of the animals gave birth to litters, but only after repeated matings. The implantation rate of the females that had received the diet plus alpha tocopherol throughout life was still practically normal at that time.

One group of 27 rats had been kept on Rockland rat diet during the first 2 months of life and thereafter on the experimental diet. At the age of 12 months, no implantations could be observed after repeated matings. When now a single dose of 6 mg alpha tocopherol acetate was offered after a single mating, litters were observed in about 2/3 of the experiments.

Discussion. Evans and Burr⁷ have discussed possible pitfalls in the evaluation of implantation data obtained from rats on vitamin E deficient diets. Failures may be caused by toxic substances in the diet or by deficiencies other than vitamin E. These sources of error can hardly explain the de-

crease in the implantation rate in these experiments, because appropriate single post mating supplements of tocopherol increased the percentage of pregnancies significantly, and breeding was normal when the diet was completed by alpha tocopherol.

Infections of the uterus and the tubes were frequently observed by us in the older rats on the deficient diet. Similar observations have previously been reported by Emerson and Evans.¹ In some instances, the uterus contained 5-10 cc thick purulent material. Purulent fistulas in the inguinal region, originating from uterine infections, were observed 4 times. No purulent infections were noted in the rats on the complete diet. While infection of the genital organs may have prevented pregnancies in some of the rats, its occurrence can be excluded in those rats which responded to single post-mating doses of alpha tocopherol. Infection of the uterus and the tubes would have interfered with both the fertilization and the implantation of the ovum.

The failure of implantation in the rats of over 8 months of age indicates that, in this age group, tocopherol is essential for the implantation of the previously fertilized ovum. The requirements appear to increase with age and there are great individual variations.

Animals that had been administered constant tocopherol supplements, had a practically normal implantation rate after one year of age; among the group receiving "massive doses" after one year or more on the deficient diet, about one-half of the rats gave birth to litters, but only when mating was carried out repeatedly; the same age group on the basic diet with single tocopherol supplements of 3-10 mg had frequent resorption gestations but only 2 litters. This indicates that the changes leading to failure of implantation are prevented but, once present, only partly cured by alpha tocopherol.

Summary. 1. The implantation rate in female rats on a purified diet containing about .1-.4 mg of tocopherols per 100 g diet was compared with the implantation rates in animals on the same diet receiving single and continuous supplements of synthetic dl alpha

tocopherol acetate.

2. Failure of implantation was observed after the eighth month in females without tocopherol supplements. With continuous tocopherol administration, the implantation rate was normal after one year. Single post-mating tocopherol supplements increased the implantation rate in older rats significantly. The requirements necessary for successful implantation in older rats vary widely and increase steeply with age.

3. Infections of the uterus and the tubes were frequently observed in the deficient rats, but not in those on the complete diet. Presence of infection does not explain the higher implantation rate after single post-mating tocopherol doses.

4. The changes leading to the failure of implantation are prevented, but once present, only partly counteracted by alpha tocopherol acetate.

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Amputation of the Canine Atrial Appendages.*

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Thrombus formation in the left atrial appendage and embolization therefrom are frequent sequelae of rheumatic mitral stenosis. Surgical excision of the thrombus-containing appendage suggests itself as a possible therapeutic approach to this problem. As a preliminary step experiments were performed to determine the effect of amputation of one or both atrial appendages in normal dogs.

Methods. The surgical procedures under anaesthesia, consisted essentially of exposure of the heart through an incision in the left fifth or sixth intercostal space, pericardiotomy, presentation of the atrial appendage with an Allis clamp, isolation of the appendage from the atrium with a right angle clamp or curved hemostat, ligation of the base of the appendage proximal to the clamp and finally amputation of the appendage. Excision of the left atrial appendage was accomplished with the animal in the left lateral position.

Electrocardiograms were obtained pre-operatively and at varying intervals during the post-operative period. The animals were sacrificed and necropsy performed 4 to 12 weeks following surgery. Eight animals were used, 5 undergoing left, 2 right and one bilateral atrial appendectomy.

Results. Seven of the 8 dogs survived the operation. The first animal developed respiratory difficulty and died 15 minutes after closure of the chest. All the surviving animals made uneventful recoveries. Serial electrocardiograms failed to reveal abnormal atrial rhythms or evidence of atrial injury except in one dog which developed transient intra-atrial block. Necropsy revealed complete endothelialization at the site of the atrial scar. No subjacent mural thrombi were found. Pericardial and pleuro-pericardial adhesions occurred.

Conclusion. Excision of one or both atrial appendages is feasible in dogs.

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