The Role of Selenium in the Placental Transfer of Vitamin E in the Rat* (33514)

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Desai and Scott (1) reported that selenium increased the retention of *d*-α-tocopherol by chicks, possibly through the formation of a selenium-containing compound acting as a tocopherol carrier in the blood. They speculated that this selenium-containing compound might function in the transfer of *d*-α-tocopherol across membranes.

Others have provided some evidence for interrelationships between selenium and vitamin E in membrane transport. Wright and Bell (2) found that dietary α-tocopherol produced a consistent but nonsignificant increase in *in vitro* uptake of ^{75}Se by erythrocytes. Further work by these authors (3) with sheep on selenium-deficient diets indicated that α-tocopherol supplementation tended to change the intracellular distribution of ^{75}Se, promoting an increase of ^{75}Se in the particulate fraction. However, α-tocopherol did not affect the transfer of ^{75}Se across the placental membranes.

This experiment was designed to determine whether selenium may play a role in the transport of α-tocopherol across the placental membrane. Transfer of tritium-labeled vitamin E to the fetuses of rats maintained on various dietary levels of selenium and vitamin E was investigated.

Methods. Twenty-four female Long-Evans rats[2] averaging approximately 275 g of body weight were fed for 3 weeks a basal diet deficient in selenium and vitamin E but containing 0.0125% ethoxyquin. They were then divided into 4 equal groups and given the following dietary treatments: (i) basal (Se and vitamin E deficient); (ii) basal + 500 IU of vitamin E/kg of diet; (iii) basal + 1 ppm of Se (2.13 mg of Na_2SeO_3/kg of diet); (iv) basal + 500 IU of vitamin E/kg + 1 ppm of Se (2.13 mg of Na_2SeO_3/kg).

The basal diet was one previously used by Bull and Oldfield (4). Distilled water was provided throughout the experiment. Following a 6-week period on their respective diets, the animals were bred. The actual time of breeding was established by daily examination of vaginal smears for sperm. On day 19 of pregnancy, 12.88 μCi (0.0299 μmoles) of DL-α-tocopherol-^3H (5-methyl-T)[3] were administered by stomach tube. This dosage was calculated on the basis of the results of Krishnamurthy and Bieri (5) on absorption, excretion, and distribution of a single dose of labeled tocopherol. In order to calculate the administered dose for each animal, the syringes used in stomach tubing were rinsed with ethanol, the washings were counted, and the appropriate corrections were applied. Twenty-four hr after the administration of labeled α-tocopherol the animals were killed and the fetuses and maternal gastrointestinal tracts were removed. These tissues were freeze-dried and then extracted for 24 hr with 95% ethanol in a Soxhlet apparatus. The alcohol extracts were reduced to a volume of 25 ml. One ml of each of these solutions was diluted to 25 ml; 0.5-ml samples of the dilute solutions were evaporated to dryness in counting vials, and 15 ml of the scintillation fluid (5 g PPO and 0.3 g of POPOP/liter of toluene)[4] was added to each vial. The contents were then counted in duplicate for 10 min in a liquid scintillation counter.

Results and Discussion. Association of the radioactivity of the fetuses with α-tocopherol was confirmed by thin-layer chromatography,

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[4] PPO = 2, 5-diphenyloxazole; POPOP = 1, 4-bis-2-(5-phenyloxazoyl)-benzene.

TABLE I. Uptake of DL-α-Tocopherol-^{3}H by Fetuses of Rats Raised on Different Dietary Levels of Vitamin E and Selenium.

Treatment and rat no.	Fetal wet wt.[a] (g)	Total fetal radioactivity (dpm)	Radioactivity per g fetal wet wt. (dpm/g)	Apparent absorbed dose retained in fetuses[b] (%)
Basal				
1	47.4 (12)	1,277,562	26,953	6.05
2	35.8 (8)	1,077,076	30,086	5.12
3	44.5 (11)	1,102,244	24,770	5.19
4	38.8 (10)	1,024,452	26,403	5.19
5	36.2 (10)	1,098,240	30,338	5.04
Mean	40.5 ± 5.2	1,115,915	27,710 ± 2423[c]	5.32 ± 0.41[c]
Basal + vitamin E				
1	52.1 (13)	764,478	14,673	3.56
2	43.4 (12)	750,178	17,285	3.56
3	36.3 (11)	824,252	22,707	4.24
4	34.8 (10)	719,004	20,661	3.65
5	40.6 (10)	795,738	19,604	3.96
Mean	41.4 ± 6.9	770,770	18,986 ± 3104	3.79 ± 0.30
Basal + selenium				
1	36.8 (9)	741,026	20,136	3.61
2	35.9 (8)	583,154	16,244	3.03
3	29.3 (7)	710,710	24,256	3.84
4	25.0 (6)	294,580	11,783	1.68
Mean	31.8 ± 5.6	582,368	18,105 ± 5335	3.04 ± 0.97
Basal + selenium + vitamin E				
1	30.2 (7)	333,476	11,042	2.08
2	32.4 (9)	454,740	14,035	3.08
Mean	31.3 ± 1.6	394,108	12,539 ± 2116	2.58 ± 0.71

[a] Number of fetuses given in parentheses.

[b] Apparent absorbed dose = radioactivity administered minus radioactivity of maternal gastrointestinal tract.

[c] These means are significantly higher ($p < 0.005$) than those for the other groups.

using the method of Bieri and Prival (6). Reproductive difficulties due to the deficiency of vitamin E in the basal diet were not anticipated (and not observed) because of the inclusion of the synthetic antioxidant ethoxyquin[5] in the rations. Crider *et al.* (7) found earlier that ethoxyquin maintained the fertility of female rats on a vitamin E-deficient diet through two generations. The breeding performance was poorest in the groups receiving supplementary selenium; mechanical inducement of pseudopregnancy during the taking of vaginal smears was the main problem. No instances of pseudopregnancy occurred in the basal group and the vitamin E-supplemented group but pseudopregnancy was observed in both groups receiving supplementary selenium. The level of selenium used (1 ppm) is not in the range (5–10 ppm) usually considered to be detrimental to female reproduction (8). The apparently increased sensitivity of the female reproductive tract to a mating response in the selenium-supplemented groups may be a factor in the improvement of ewe fertility noted in New Zealand when selenium-deficient animals were administered selenium (9).

Pertinent data on the transfer of labeled α-tocopherol to the fetuses are given in Table I. Greatest transfer occurred in the basal

[5] Monsanto Chemical Co., St. Louis.

group, in which the dpm per gram of wet fetal weight was significantly higher ($p<$ 0.005) than for the other groups. The higher transfer was also reflected in the proportion of the dose found in the fetuses. The percentage of the apparent absorbed dose (dose administered minus labeled material in the maternal gastrointestinal tract) in the fetuses was significantly higher ($p<0.005$) in the basal group than in the others. In comparing the basal and selenium-supplemented groups, it is apparent that supplementary selenium did not increase the transfer of α-tocopherol but in fact significantly reduced it.

Two interpretations may be advanced for the reduction in the transfer of α-tocopherol to the fetuses of the selenium-supplemented rats. First, it may be hypothesized that the presence of selenium in the diet reduces the fetal requirement for α-tocopherol. This concept is supported by the observation that fetal uptake of labeled α-tocopherol was approximately equal when the basal diet was supplemented individually with vitamin E or selenium, and was lowest when both selenium and vitamin E were supplemented coincidentally. Selenium, may, therefore, have a sparing effect on the tocopherol requirement of the rat fetus. Such effects have been reported; for example, Desai and Scott (1) observed that dietary selenium reduced the level of vitamin E required for prevention of nutritional muscular dystrophy in chicks. Alternatively, it may be suggested that since selenium has been reported to promote retention of α-tocopherol in the tissues of the rat (10), less of the administered dose might have been available for fetal transfer in the groups receiving supplementary selenium.

Summary. Transfer of tritium-labeled α-tocopherol across the placental membrane was significantly lower ($p<0.005$) in rats receiving dietary selenium supplementation than in a selenium- and vitamin E-deficient group. Approximately equal fetal uptake of radioactivity was observed in groups receiving either supplementary selenium or vitamin E, while the lowest placental transfer occurred in rats receiving coincidental dietary supplementation with both selenium and vitamin E. Whether these differences in transfer of α-tocopherol to the fetuses relate to a sparing effect of selenium for vitamin E in the fetal tissue, or are due to variations in the amount of maternal tissue retention of the administered dose, thus limiting the amount available for fetal uptake, remains to be determined.

1. Desai, I. D. and Scott, M. L., Arch. Biochem. Biophys. **110**, 309 (1965).

2. Wright, P. L. and Bell, M. C., Proc. Soc. Exptl. Biol. Med. **114**, 379 (1963).

3. Wright, P. L. and Bell, M. C., J. Nutr. **84**, 49 (1964).

4. Bull, R. C. and Oldfield, J. E., J. Nutr. **91**, 237 (1967).

5. Krishnamurthy, S. and Bieri, J. G., J. Lipid Res. **4**, 330 (1963).

6. Bieri, J. G. and Prival, E. L., Proc. Soc. Exptl. Biol. Med. **120**, 554 (1965).

7. Crider, Q. E., Alaupovic, P., and Johnson, B. C., J. Nutr. **73**, 64 (1961).

8. Underwood, E. J., "Trace Elements in Human and Animal Nutrition," 2nd ed. Academic Press, New York (1962).

9. Hartley, W. J. and Grant, A. B., Federation Proc. **20**, 679 (1961).

10. Witting, L. A., Harmon, E. M., and Horwitt, M. K., Fed. Proc. **26**, 475 (1967).

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