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Specific Effect of Selenium Deficiency on Rat Sperm[1]

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ABSTRACT

Reproductive disorders in Se-deficient male rats from Se-adequate dams are described. After 4, 11 and 12 months on the Se-deficient (<0.02 ppm) diet, small, but sometimes significant, reductions in body and testicular weights were observed. Sperm morphology and motility appeared to be normal after the 4 month interval, but 50% of the animals in the 11 and 12 month interval groups produced sperm with impaired motility and a characteristic midpiece breakage. Because the morphologic anomaly does not appear to be correlated with effects on body and testicular weights, the effect of selenium on sperm is thought to be rather specific. The possible involvement of the Se-dependent enzyme glutathione peroxidase (GSH-P_X) in maintaining the integrity of the sperm membrane is discussed.

INTRODUCTION

Selenium (Se) has been implicated in the process of reproduction, at both ends of the supply spectrum. Rosenfeld and Beath (1954) reported that excess selenium in the diet impaired fertility in female rats, but that the male did not seem to be affected. Later, Andrews et al. (1968) demonstrated in their extensive New Zealand studies that Se deficiency seriously impaired reproduction in sheep. Hartley (1963) suggested that the latter problem was related to fetal development rather than interference with conception since fetal resorption occurred in the Se-deficient ewes. Similarly, Jensen (1968) demonstrated reduced hatchability and chick viability caused by Se deficiency in Japanese quail. Up to this point, the effect of Se deficiency on reproduction seemed to be oriented toward the female of various species.

Wu et al. (1969, 1971) utilizing laboratory rats, found that Se deficiency also affected male reproductive functions. Their results showed that Se deficiency in male offspring from Se-deficient female rats produced sperm with impaired motility and a unique type of midpiece abnormality. It was found that dietary supplementation with α-tocopherol or other antioxidants did not alleviate these symptoms of Se deficiency (Wu et al., 1973). These findings suggest that Se plays a specific role in maintaining the structural and functional integrity of the sperm cell. However, in these studies, the body and testicular weights of the Se-deficient rats were greatly reduced and it was difficult to determine whether the formation of abnormal sperm in Se-deficient rats was due to a direct effect of Se on sperm morphology or was caused indirectly via the element's broader influence on growth. The present experiment was conducted to elucidate further the role of Se on spermatozoa of the rat, using offspring produced from parent animals maintained on a Se-adequate diet.

MATERIALS AND METHODS

Two separate experiments were conducted. In each, rats of the Long-Evans strain, born to dams fed a Se-adequate diet, were placed at weaning into groups and fed variations of a basal diet formulated to contain less than adequate amounts (<0.02 ppm) of Se. The composition of the basal diet is given in Table 1.

Experiment 1

At weaning 24 male rats were assigned randomly to 2 treatment groups of 12. Group 1 was fed the basal diet (Table 1) deficient in Se (<0.02 ppm), while Group 2 received the basal diet plus 0.5 ppm selenium, supplied as sodium selenite.

The animals were individually housed in stainless steel cages without bedding and were given distilled water *ad libitum*. Feed intake and growth rate of each animal were monitored weekly. Six animals from each group were killed after 4 months and the survivors were killed after 12 months on experiment. The testes and epididymides from each animal were removed, weighed and prepared for semen evaluation and histological studies.

For histological studies, tissue sections were cut at a thickness of 5 or 10 μm and stained with Mallory's

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TABLE 1. Composition of basal diet (Se-deficient).

Se-deficient basal ration	%	Vitamin mix (make up to 100 g with sucrose)	
Torula yeast	30.0	Vitamin A	20,000 IU
Sucrose	59.0	Vitamin D	4 mg
Alphacel	1.8	Menadione	1 mg
Lard	4.0	Thiamine	25 mg
Methionine	0.2	Pyridoxine	12 mg
Salt mix[a]	4.0	Niacin	150 mg
Vitamin mix	1.0	Ca-pantothenate	80 mg
		Riboflavin	25 mg
		Folic acid	20 mg
		Biotin	2 mg
		B-12 (1% trituration)	1 mg
		Vitamin E (DL-α-tocopherol)	300 mg
		Choline-Cl	7.5 g

[a]J. Nutr. 14,273 (1937).

triple stain (Humason, 1967). Semen smears were prepared by suspending material recovered from the cauda epididymidis in physiological saline. Relative number and activity of sperm were examined with a light microscope at X 400 magnification at 37°C and graded from 0–10, with 10 representing the highest motility. The morphology of the sperm cells was evaluated using both the light microscope and electron microscope (EM). For EM studies, the sperm cells were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7, at 5°C for not less than 24 h. They were then dehydrated with ascending concentrations of ethanol and ethanol-trichlorotrifluoroethane and dried by the critical point technique (Anderson, 1951). The specimens were then shadowed with chromium at a 30° angle and examined with a Philips EM-300 electron microscope (Philips Electronic Instruments, Inc., Mahwah, NJ).

Experiment 2

At weaning, 24 male rats were assigned randomly to 2 groups of 12. Twelve males (Group 3) were fed the Se-deficient basal diet while the other 12 (Group 4) received the basal ration supplemented with 0.4 ppm selenium, as Na_2SeO_3, as in the previous experiment. These animals were killed after 11 months on experiment. Histological preparations of testes and epididymides and semen evaluations were carried out as described for Groups 1 and 2.

RESULTS AND DISCUSSION

In contrast to the severe reduction in body weight (33%) and testicular weight (41%) in Se-deficient rats born to Se-deficient dams in previously reported experiments (Wu et al., 1973), the body and testicular weights of the Se-deficient offspring from Se-adequate rats in the present study were only slightly reduced as compared with controls. (Reductions were 7–15% and 4–16%, respectively, for the body and testicular weights.) Four months or 12 months after the initiation of the experiments, as shown in Table 2, the difference in body weight in rats on Se-deficient and Se-adequate diets was not significant. However, in the 11 month group, the body weights of Se-deficient animals were significantly lower than the body weights of animals on diets supplemented with Se. The differences in testicular weights between Se-deficient and Se-adequate animals were significant for rats killed at 4 or 11 months after the initiation of the experiments.

Spermatozoa from rats on Se-deficient diets for 4 months were actively motile and showed no midpiece abnormality. After 11 or 12 months of Se deficiency, however, half of the animals produced sperm that was completely nonmotile. These sperm also showed, under the light microscope, evidence of midpiece abnormality (Fig. 2) that we consider to be characteristic of Se deficiency. Electron microscopy confirmed that the damage was oriented toward the sperm midpiece and appeared to affect the membrane system, at least the outer membrane, of the sperm structure (Fig. 3). Damage in some cases was extensive, but did not appear to affect the head portion of the sperm cell (Fig. 4). This type of midpiece abnormality is very specific and has not been described in sperm cells except as reported in our previous study in Se-deficient rats (Wu et al., 1973). The present study helps to define the relationship of Se deficiency to sperm

TABLE 2. Body and testicular weights and spermatozoan morphology and motility from rats on Se-deficient and Se-adequate diets.

Rations	No. rats	Duration (month)	Body wt. (g)	Testes wt. (g)	Sperm motility (0–10)	No. of rats with sperm midpiece abnormality
Experiment 1						
Basal	6	4	452 ± 5.58[a]	3.29 ± 0.06*	6.2	None
Basal + Se	6	4	470 ± 18.37	3.55 ± 0.11	6.2	None
Basal	4[c]	12	476 ± 17.08	3.03 ± 0.30	2.5	2/4[b]
Basal + Se	4[c]	12	494 ± 12.33	3.57 ± 0.07	5.8	None
Experiment 2						
Basal	10[c]	11	456 ± 15.94	3.07 ± 0.09**	3.5	5/10[b]
Basal + Se	12	11	543 ± 17.66*	3.50 ± 0.08	8.0	None

[a]Mean ± SEM. Student's t test was used for statistical comparisons (Petersen, 1975) between Se-deficient and supplemented groups.

[b]Sperm were nonmotile and showed midpiece abnormality characteristic of Se deficiency (Wu et al., 1973).

[c]Two rats died in each of these groups.

*$P<0.05$.

**$P<0.01$.

development, since the abnormality characteristic of Se deficiency occurred in rats which were less Se-deficient, having been born to Se-adequate mothers. Under such conditions, the spermatozoa again appeared to be target cells in which the unique Se-deficient syndrome occurred with comparatively slight reduction in body and testicular weights.

In experiment 1, the average body weights of Se-deficient rats in both of the 4 and 12 month groups were 96% of those in the Se-supplemented groups. In spite of this similar magnitude (4%) of reduction in body weights, the rats of the 4 month group did not show sperm abnormality while 50% of those in the 12 month group did show characteristic sperm midpiece abnormality. Body weight reduction, *per se*, therefore does not appear to be the causative agent for this type of sperm abnormality.

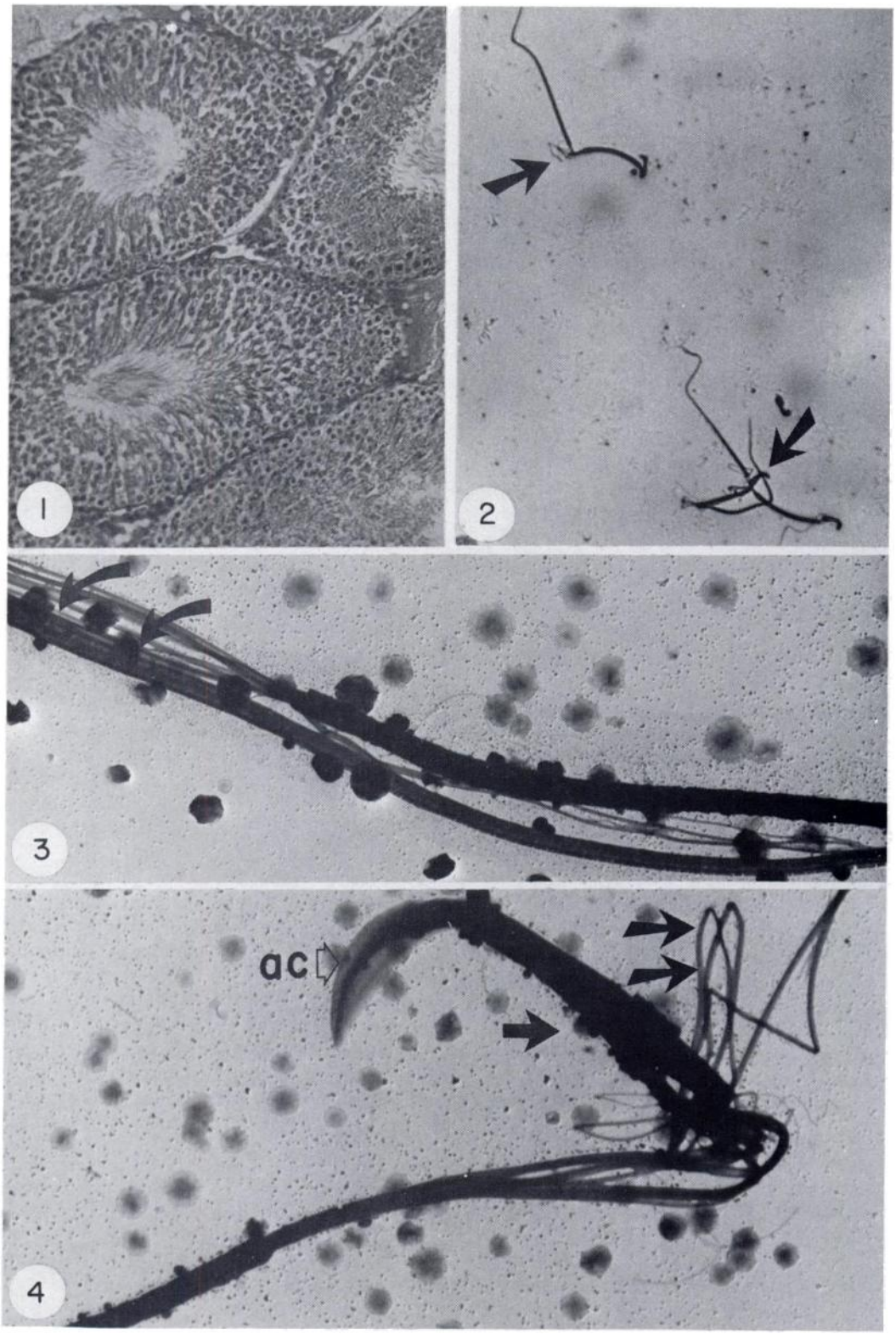
Neither is there evidence indicating the sperm anomaly associated with Se deficiency is caused by the reduction of testicular weight. In rats receiving the Se-deficient ration for 4, 11 and 12 months, there were reductions in testicular weights (Table 2) averaging 7, 12 and 15%. The testes from all 26 rats on Se-deficient diets showed no gross abnormality and histological sections indicated active spermatogenesis. Furthermore, in the 12 month group of experiment 2, the testicular weights of the 4 rats on Se-deficient diets were 2.15, 3.21, 3.33 and 3.45 g, respectively, but those that produced sperm with the midpiece abnormality were the 2 that had heavier testicular weights (3.33 and 3.45 g). In experiment 2, although the mean testicular weight was significantly greater in the Se-adequate group, there were several instances where Se-deficient rats showing midpiece abnormality had heavier testes than some of the Se-adequate rats. Reduction of testicular weight in the Se-deficient rats does

FIG. 1. Section of testis from rat fed Se-deficient diet for 12 months. Note the presence of active spermatogenesis.

FIG. 2. Sperm from rat on Se-deficient diet for 12 months. Note the midpiece abnormality (↓). ×220.

FIG. 3. Electron micrograph of sperm tail from Se-deficient rat. Note the abnormal separation of the tail filaments (↓↓). ×4950.

FIG. 4. Electron micrographs of sperm tail and head. Note the fraying of the tail fibril (↓↓), but that the head (↓) appears to be normal with its intact acrosome (ac) clearly identifiable. ×4950.



not therefore seem to be the direct cause of sperm midpiece abnormality.

It is evident that the absence of gross abnormality as well as the presence of active spermatogenesis in Se-deficient rats cannot rule out all possible subtle testicular changes that may influence sperm morphology. However, unless any such subtle change has been positively identified, it appears to be more convincing to propose that Se plays a direct role on sperm structure rather than an indirect role via its effects on somatic and gonadal development and growth.

The results clearly demonstrate that Se deficiency, in addition to its known effect on reproduction in the female rat, has an adverse effect on reproduction in the male rat. This observation agrees with the report from McCoy and Weswig (1969) who found that 5 out of 8 male rats from a second generation population fed a low Se ration produced immotile spermatozoa with "separation of heads from tails." The electron microscopic demonstration of the site of damage at the sperm midpiece, supports the earlier observations with the light microscope (Wu et al., 1969, 1971, 1973) and those of Brown and Burk (1973) who administered ^{75}Se and showed by autoradiograph that the ^{75}Se was concentrated at the sperm midpiece.

Although it is not possible, from the results of this study, to define the exact mechanism involved in this Se deficiency-induced sperm anomaly, one can speculate that selenium is influencing the surrounding membrane to produce its effect on the structural and functional integrity of sperm cells. It is well known that in white muscle disease, or Se-responsive nutritional muscular dystrophy, there is damage to cellular membranes which causes some of the intracellular enzymes to leak out into the extracellular fluid (Blincoe and Dye, 1958). In the Se responsive "exudative diathesis" in chicks, there is a massive loss of fluid from the capillaries into subcutaneous pockets in the breast and abdomen. Such lesions lead to elevated levels of glutamicoxalacetic transaminase (GOT) in plasma. The presence of this enzyme system in seminal plasma and in ejaculated sperm (Flipse, 1969) may provide further useful information on the question of how Se may affect sperm viability.

Se is also known to function as part of the gluthathione peroxidase (GSH-Px) enzyme system (Rotruck et al., 1973). The levels of GSH-Px have been shown to be higher in bovine seminal plasma (3345 ± 207 EU/ml) than epididymal sperm (534 ± 98) (Brown and Senger, 1977). More recently, Smith et al. (1978) suggested that Se may perform a dual function: in bovine seminal plasma as an essential component of GSH-Px and in spermatozoa as a structural component.

Noguchi et al. (1973) proposed that the membrane protecting effect of Se was one of preventing oxidative damage to the membrane lipids and this may be operating in male reproductive cells or tissues. Vitamin E as a free radical scavenger, will prevent peroxide formation. Se, via glutatione peroxidase, may eliminate peroxides before cell damage ensues. Such a proposed mechanism seems to fit well with the report of Brown and Burk (1973) who found a high uptake of ^{75}Se by the testis and epididymis and a high concentration of ^{75}SE in the sperm midpiece. The mitochondria of the testis contained more than twice as much ^{75}Se as those of the liver. These findings suggest that Se is an essential constituent of the sperm cell. Its presence in seminal plasma appears to protect the sperm membrane from damage by metabolic free radicals. Deficiency of this element could theoretically lead to the loss of cellular enzymes (Shull et al., 1978), membrane damage and dysfunction of the cell.

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