Effect of Pollack Liver Oil in Rat Testes

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Rat testes are studied 1, 3, 6, and 12 month after addition of pollack liver oil (0.1 g/kg body weight) to daily ration. A 3-month feeding of the fish-oil-enriched diet leads to degenerative changes of spermatozoa and spermatids in 8-12% convoluted tubules. Continuation of the treatment to 6-12 months induces damage to spermatocytes and spermatogonia in 30-60% tubules. The observed changes can be attributed to consumption of high doses of retinol, a constituent of pollack liver oil.

Key Words: *testes; spermatogenesis; pollack liver oil*

Fish oil is widely used in the fishing industry, veterinary, and medical practice as an important source of polyunsaturated fatty acid (PUFA) and vitamin A. Pollack liver oil (PLO) is particularly rich in these nutrients; therefore, it is successfully used in the treatment of cardiovascular and respiratory diseases and as an immunocorrector.

However, vitamin A, a constituent of PLO, has an adverse effect on some organs, primarily on male reproductive system, which results in aspermia and infertility [1,3,4,8,9].

The present study examines structural elements of rat testes during long-term consumption of PLO.

MATERIALS AND METHODS

The study was carried out on the testes from 120 mature albino rats weighing 200 g. Control animals were fed standard chow without supplements (control I) or supplemented with 0.1 g/kg sunflower oil (SO, control II). Experimental animals were given standard chow supplemented with 0.1 g/kg PLO containing 45% PUFA (21% eicosapentaenoic and 22% docosahexaenoic acids) and 250 U vitamin A (retinol). The content of PUFA in PLO (oil quality) was analyzed by measuring the acidic, hydroperoxide, and iodine numbers. The residual pesticide content did not exceed the maximum permissible level, and the content of toxic elements was within the standards established by the USSR Ministry of Health. The rats were decapitated 1, 3, 6, and 12 months after the start of experiment. The testes were fixed in Stieve fluid and 10% formalin, embedded in paraffin, and 6-μ thick sections were then cut and stained with hematoxylin and eosin. The mean area of seminiferous tubules, the number of affected convoluted tubules, the index of spermatogenesis, and the number of spermatogonia and spermatocytes were evaluated as described elsewhere [5]. The data were processed statistically using ANOVA tests.

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RESULTS

The structure of testes in rats fed PLO or SO during 1 month did not differ from that observed in intact animals (control I). In all cases the testicular lobules were packed with concentric or ovoid cross-sections of seminiferous tubules. Three or four generations of spermatogenic cells at different stages of differentiation were seen in the tubules (Fig. 1, *a*). The regular cell arrangement corresponded to the stages of spermatogenesis. Sertoli cells with a wide basis and small apex were adjacent to the lamina propria of the tubule. Large glandulocytes (Leydig cells) clustered in the connective tissue between the seminiferous tubules, usually around blood vessels.

A 3-month intake of an SO-enriched diet produced no marked changes in the structure of spermatogenic cells, as well as in Sertoli and Leydig cells. The number of tubules with desquamated germ cells

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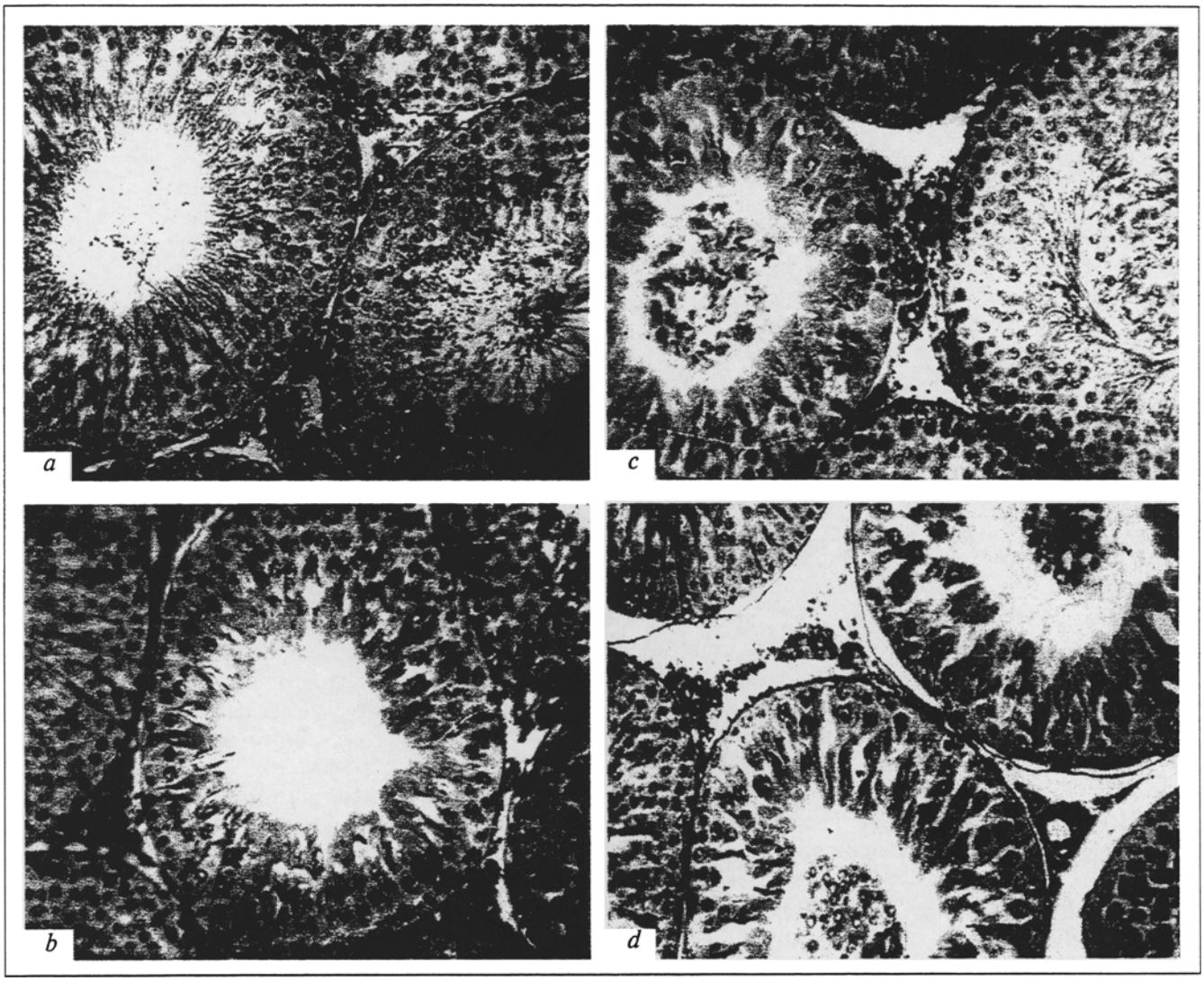

Fig. 1. Structure of convoluted tubules in testes from control rats (*a*) and animals treated with pollack liver oil for 3 (*b*), 6 (*c*), and 12 (*d*) months. Hematoxylin and eosin staining. ×160.

increased by 2-3%, while the mean cross-section area of the tubules decreased by 3-5% ($p>0.05$). More pronounced alterations were noted in the testes from animals fed PLO for 3 months: desquamated germ cells were seen in 18-20% tubules (Fig. 1, *b*), the mean cross-section area decreased by 9-12%, while the mass of testes increased by 30% ($p<0.05$). Clear-cut microscopic changes were seen in 8-12% convoluted tubules. No spermatids and spermatozoa were found in these tubules, which attests to the loss of spermatogenic function [1,7]. The total number of spermatocytes and spermatogonia slightly decreased (by 5-7%); spermatocytes with homogeneous intensely stained cytoplasm frequently occurred. Small round cavities in the tubular wall formed in the place of degenerated spermatocytes. The structure of most spermatogonia was preserved; mitotic figures were seen in numerous cells. The index of spermatogenesis reduced by 10% in comparison with control I and control II due to depletion of the tubules with spermatogenic cells. The structure of Sertoli and Leydig cells remained practically unchanged.

Continuation of the SO-enriched diet to 6 months had no marked effect on the number and arrangement of testicular cells. Most tubules were characterized by active spermatogenesis. No significant changes were found in the number of convoluted tubules with desquamated germ cells, the mean cross-section areas of the tubules, and the index of spermatogenesis.

In rats maintained for 6 months on a PLO-enriched diet, all studied parameters significantly differed form both control I and control II. The number of seminiferous tubules with desquamated germ cells increased considerably (6-14-fold) in comparison with the control, their cross-section area being one-third decreased. The tubules with pro-

nounced destructive processes constituted 25-30% (Fig. 1, *c*). Spermatids were absent; large ovoid structures with degenerating pyknotic nuclei, and seminal globules frequently occurred. The total number of spermatocytes considerably dropped (35-40%), some tubules contained no spermatocytes. Among preserved cells, spermatocytes with dense nuclei and homogeneous basophilic cytoplasm were seen. Spermatogonia and Sertoli cells were also involved into the destructive process. The total number of spermatogonia decreased by 17-20% ($p<0.01$), which was accompanied by a decreased number of mitoses. The index of spermatogenesis dropped by 38% in comparison with intact controls due to depletion of the tubules with spermatogenic cells. Leydig cells remained visually unaffected, their number being somewhat increased.

In animals receiving SO-enriched diet for 12 months, we observed an increase in the number of seminiferous tubules with desquamated germ cells and reduction in the mean cross-section area of the tubules by 10 and 14%, respectively, in comparison with control I ($p>0.05$). The structure of spermatogenic and Leydig cells remained unchanged; the total number of altered convoluted tubules did not exceed 3-5%.

A 12-month treatment with PLO induced dramatic changes in the studied parameters (by 30-40%) in comparison with those observed in rats fed PLO for 6 months. Tubules with destructive changes constituted 55-60%.

Light microscopy revealed various morphological changes in the seminiferous epithelium. Apart from tubules with preserved cell structure, we observed tubules with partial or complete cell degeneration (Fig. 1, *d*). Seminal globules were sometimes seen; however, the occurrence of abnormal spermatocytes were rather high in the tubular lumen. De-epithelialized fragments of the seminiferous tubules were found with flattened Sertoli cells adjacent to the lamina propria and contacting with solitary spermatogonia. An increased number of large Leydig cells was seen in the interstitial tissue around blood vessels.

Thus, a long-term intake of PLO results in destruction of the spermatogenic cells in some seminiferous tubules. The number of affected tubules and the intensity of degenerative processes in germ cells increase along with the duration of PLO treatment. However, highly differentiated spermatogenic cells are highly resistant to this effect of PLO. These changes impair the spermatogenic function of the testes and result in hypospermia and reduced fertility of animals [2,6,9]. Negative effect of PLO on spermatogenic cells is probably due to a high content of vitamin A. Similar and even more pronounced changes in the convoluted tubules induced by the corresponding doses of retinol or retinoic acid were also observed by other investigators [1,3,8]. Thus, to prevent the adverse effect of PLO on the testes the intake of this nutrient should be limited by 1-3 months, which ensures minimal damage to the seminiferous tubules.

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