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Effect of Pollak Liver Oil on Morphology and Function of Rat Testes

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Rat testes are studied 1 month after addition of physiological and excessive doses of pollak liver oil to diet. High doses of pollak liver oil cause degenerative changes in the spermatogenous epithelium, primarily in high differentiated cells (spermatozoa, spermatids, and spermatocytes I). In a dose of 1 g/kg pollak liver oil causes damage to connective tissue of convoluted tubules, which results in aspermia and sterility.

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

Polyunsaturated fatty acids (PUFA), components of sea-fish oil, are effectively used in the treatment of hypercholesterolemia, hyperlipidemia, and other dyslipidemias [1-3,5]. Alaska pollak (*Theragra chalcogramma*) is an important source of PUFA and vitamin A. Modern (low-temperature) technology makes it possible to obtain pollak oil preserving its biological and nutritional value.

However, the validity of fish oil as a nutritional supplement is in doubt, since high doses of pollak liver oil (PLO), which are known to be most effective in treating cardiovascular and respiratory diseases, have adverse effects on other organs [6].

In the present study we explore the effect of high doses of PLO on the testes.

MATERIALS AND METHODS

The study was carried out on the testes from 100 mature albino rats weighing 200 g. Group 1 animals were fed standard chow. Group 2 rats were additionally given 0.1 g/kg PLO (physiological dose). Group 3 and 4 animals received 0.3 and 1.0 g/kg PLO, respectively. PLO contained 45% PUFA and 250 U vitamin A. 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 Ministry of Health. The rats were decapitated 1 month after the start of the experiment. The testes were fixed in Stieve fluid and 10% formalin, embedded in paraffin, and 6-μ-thick sections were 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 assessed [5]. The data were processed statistically and compared using ANOVA tests.

RESULTS

In intact animals (group 1), testicular lobules are packed with concentric or ovoid cross-sections of seminiferous tubules. Three or four generations of spermatogenic cells at different stages of differentiation are seen in the tubules (Fig. 1, *a*). The regular cell arrangement corresponded to the stages of spermatogenesis. Sustenocytes (Sertoli cells) with a wide basis and small apex are adjacent to the lamina

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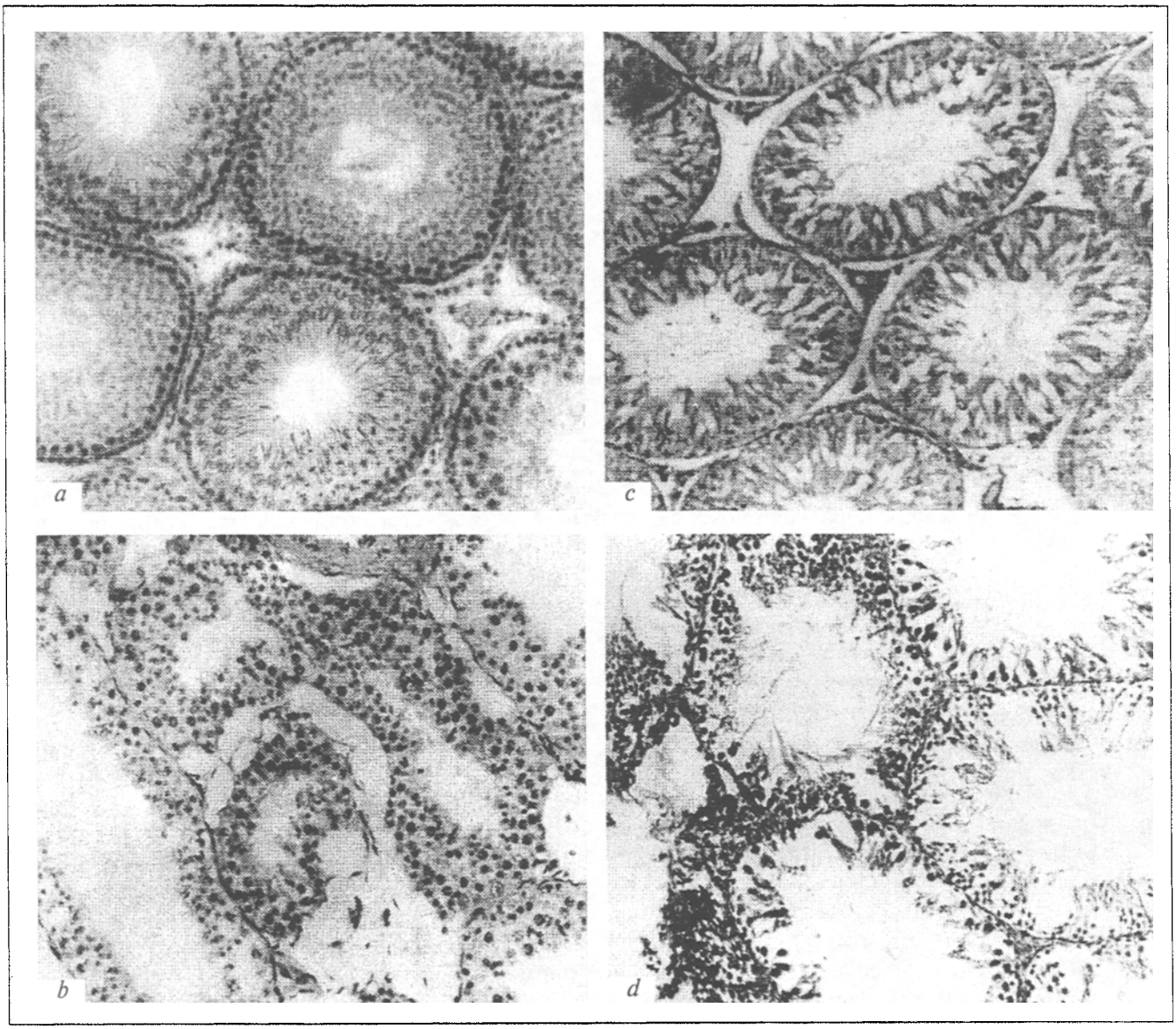

Fig. 1. Testicular convoluted tubules in intact animals (*a*) and rats treated with pollak liver oil in doses of 0.3 (*b*, *c*) and 1 g/kg (*d*). Hematoxylin and eosin staining, ×80.

propria of the tubule. Large glandulocytes (Leydig cells) are clustered in the connective tissue between the convoluted tubules, primarily around blood vessels.

In rats receiving physiological doses of PLO (group 2) for 1 month, the number of tubules with desquamated germ cells increases, but the majority of tubules have regular structure of the spermatogenous epithelium and Sertoli cells. Small groups of interstitial cells lay between the tubules; their number and structure do not differ from the control.

A diet containing 0.3 g/kg PLO increases testicular and total body weight due to accumulation of subcutaneous fat. Microscopy showed that some convoluted tubules retain regular structure, but about one third of them undergo destructive changes of various degree (Fig. 1, *b*, *c*). In some tubules, spermatogenous epithelium consists of 3-4 layers corresponding to differentiation stages; the cells do not differ from the control, but numerous desquamated immature germ cells are seen in the lumen. There are some tubules with 1-2 cell layers, adjacent to the basal membrane. Spermatids and most spermatocytes disappear or undergo degenerative alteration. Small round cavities appear in the tubular wall in the place of degenerated spermatocytes (Fig. 1, *c*). Some spermatocytes have homogenous intensely stained cytoplasm. Single abnormal spermatogonia and spermatocytes frequently occur in the lumen of convoluted tubules. The index of spermatogenesis and the area of convoluted tubules significantly decrease by 18% ($p<0.05$). The number of spermatogonia and

spermatocytes decrease by 23 and 30%, respectively ($p<0.01$). The number of tubules with desquamed epithelium increases 15-fold in comparison with intact animals and 6.5-fold in comparison with group 2.

On the other hand, mitotic figures are found in remaining cells, the structure of Sertoli and Leydig cells remaines practically unchanged, attesting to potential recovery of spermatogenesis. The number of interstitial cells slightly increases.

In group 4 animals spermatogenesis completely stopped in 80-85% tubules because of atrophy of the spermatogenous epithelium (Fig. 1, *d*). These tubules have large lumen and thin walls with 1-2 generations of spermatogenic cells. Compared with groups 1 and 2, the mean area of convoluted tubules decreased by 25 and 18%, respectively ($p<0.05$). Some tubules contain no spermatids and spermatocytes and only solitary spermatogonia and Sertoli cells adjacent to the basal membrane. The number of spermatogonia and index of spermatogenesis decreases by 35 and 53% ($p<0.05$), respectively. Some Sertoli cells have regular structure, while others lose most of their cytoplasm.

In some tubules, spermatogenous epithelium is completely absent and the walls are lined with expanded syncytium of Sertoli cells; loose adventitia of convoluted tubules somewhere lacks myocytes and fibroblasts. These tubules are usually surrounded by enlarged interstitial cells.

Thus, excessive doses of PUFA induce degenerative changes in spermatogenous epithelium, which can result in aspermia and sterility in experimental animals. These changes involve primarily high differentiated cells (spermatozoa, spermatids, and spermatocytes I). Spermatogonia and Sertoli cells are less susceptible to PLO. Most interstitial cells preserve normal structure. Intake of 1 g/kg PLO causes pronounced structural alteration in testes, such as atrophia of spermatogenous epithelium and destruction of the adventitia of convoluted tubules.

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