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Testicular Tumors in the Teleost (*Xiphophorus helleri*) Receiving Sesame Oil.

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Sesame oil is often used as a vehicle for fat-soluble endocrine preparations which gives the protracted action for free hormones. Pinkerton¹ suggested the use of this oil for roentgenologic purposes as it appears to have no reaction when introduced into the lungs. There are increasing evidences, however, that it may not be inert under some other conditions.²⁻⁸ The possibility of induction of subcutaneous sarcomas when this oil is used as a hormone-solvent in injections has been indicated by Voegtlin.⁹ Recently, Conrad *et al.*¹⁰ reported a case of sesame oil tumors in the subcutaneous tissue of the arms of a woman who was treated with estrogen dissolved in the oil. Steiner *et al.*¹¹ found that overheated (350°C) sesame oil is carcinogenic, inducing 3 sarcomas in 9 mice that lived for more than one year.

In connection with another study of endocrine activities in the teleost, *Xiphophorus helleri*, a small control group of animals was injected with sesame oil only. Upon microscopical examination, a malignant testicular tumor of the interstitial cells had developed in two specimens and later a nodular hyperplasia of interstitial cells was noted in another.

The present paper is concerned with studies of these interstitial cell tumors together with notes on some other effects of sesame oil injections, in an extended series of experiments.

Materials and Methods. In one series, 16 adult males were used, 8 of which served as untreated controls and the others were injected retroperitoneally with a weekly dosage of 0.02 cc sesame oil. Five animals received 4 injections each, and the other 3 animals were each subjected to 6 injections. They were killed 2 weeks after the last injection. In the second series 44 adult males were used, 12 of which were untreated controls. Thirty-two fish were each injected with 0.02 cc of sesame oil at intervals of 15 days. Six of them each received 27 or more injections. In both series, the fish were kept individually in separate aquaria. The visceral organs were removed as one mass at autopsy and fixed in Bouin's or Zenker's fluids. Serial sections were stained either with hematoxylin and eosin or Mallory's connective tissue stains.

Observations. Generally upon autopsies of the oil-injected fish a few droplets of oil were found in the peritoneal cavity. The omentum, which in pisces is usually undeveloped, was found to be filled abundantly with fat and small fat-nodules in prolonged treated fish. The lymph plexus also was well distended with fat. Histological examination revealed that large macrophages characterized by brownish yellow lipid material were present in the peritoneum, spleen, mesonephros, and other visceral organs. Evidences of degenerative hepatitis and fatty liver were seen in some specimens. Normally the white, elongated testis is located in a position posterior to the intestine, just beneath the dorsal peritoneum. The posterior part of the testis generally shows more or less complete bilaterality, while the anterior part is small and single. Each lobe of the bifurcated testicle consists of a centrally

¹ Pinkerton, H., *Arch. Path.*, 1928, **5**, 380.

² Pollia, H. A., *Radiology*, 1937, **29**, 683.

³ Spurr, C. L., and Kochakian, C. D., *Endocrinology*, 1939, **25**, 782.

⁴ Tobin, C. E., *Endocrinology*, 1941, **28**, 419.

⁵ Bruce, R. A., and Tobin, C. E., *Endocrinology*, 1940, **27**, 956.

⁶ Clausen, F. W., *Endocrinology*, 1940, **27**, 989.

⁷ Stein, K. T., and Allen, E., *Anat. Record*, 1942, **82**, 1.

⁸ Crafts, R. C., *Endocrinology*, 1942, **31**, 142.

⁹ Voegtlin, C., *J. Nat. Cancer Inst.*, 1942, **2**, 309.

¹⁰ Conrad, A. H., Conrad, A. H., Jr., and Weiss, R. S., *J. Am. Med. Assn.*, 1943, **121**, 237.

¹¹ Steiner, P. E., Steele, R., and Koch, F. C., *Cancer Research*, 1943, **3**, 100.

placed sperm duct which usually contains many spermatophores, and tubules within which are seen the germ cells. Germ cells of the same spermatogenic stage are clustered together forming numerous cysts.¹² The interstitial cells as seen in sections are ordinarily very scanty and scattered, with blood vessels among the sperm ducts and tubules.¹³

The first tumor was observed to occur in a fish which had received only 6 injections. This testis was slightly enlarged, the bifurcated halves now fuse so that in transverse sections the gonad appeared single. A large portion of the sperm tubules were now replaced by the tumor cells with an indefinite limiting margin of fibroblasts. Thus the tumor cells merged with the surrounding interstitial cell hyperplasia and the remaining spermatocysts. The partially atrophied sperm ducts either contained atypical released spermatozoa or were entirely empty. The tumor cells which were identified as interstitial cells were polygonal in shape with a single nearly rounded vesicular nucleus. Many of these cells, however, were vacuolated and only rarely showed mitotic figures. Blood vessels were rare and small foci of yellow-colored macrophages were observed among the tumor cells, and were infiltrated into the mesorchium but no other metastases were found.

In the second series of experiments, a malignant testis tumor occurred in a fish which had received 23 injections. The testis was a single, greatly enlarged mass and the tumor was similar to that just described. Many small empty cysts which marked the atrophic sperm-tubules were often surrounded by fibroblasts. As layers of fibroblasts inclosed the lumen of sperm ducts which contained mainly the debris of necrotic epithelial cells, the whole structure appeared like a lymph nodule. Blood vessels and macrophages were more abundant than in the previous case. Mitosis of tumor cells was rare and many cells showed foamy cytoplasm or necrosis. No metastases except in the mesorchium were seen. Cysts containing necrotic

cells, macrophages, and lymphocytes were found within the lobules of the dispersed pancreas on the fatty mesentery.

In the testicle of a fish which had received 23 injections, a considerable number (7) small nodules of interstitial cell hyperplasia were discovered. These nodules were located either on the periphery or near the sperm ducts. The largest nodular mass was 0.2 mm in diameter. Each nodule consisted of many foamy tumor cells and was well demarcated from the rest of the testicular tissue. Cysts of necrotic cells were again seen in the pancreatic lobules of this fish and a mild degree of fatty liver was also observed.

Discussion. Jemerin¹⁴ reported a case of interstitial-cell tumor of the testicle in man and critically reviewed the previous records of the similar hyperplasia and neoplasia in man as well as in horses and dogs. Recently, several additional cases of interstitial-cell tumors of the human testicle were noted by Bonser and Hawksley¹⁵ and Warren and Olshausen.¹⁶ The latter workers classified the growths into 3 groups: hyperplasia, local nodular tumor, and malignant tumor. Testicular tumors appear to occur more frequently in animals other than man.¹⁷ Innes¹⁸ examined testicle-tumors from 1 calf, 2 horses, and 49 dogs, of which 3 cases were identified as the interstitial-cell tumors. Besides, he observed 12 cases of nodular hyperplasia of interstitial cells in old dogs. It is well known that both benign and malignant tumors of interstitial cells may be induced in certain strains of mice when treated with estrogens.¹⁹ Thus far

¹⁴ Jemerin, E. E., *Arch. Surg.*, 1937, **35**, 967.

¹⁵ Bonser, G. M., and Hawksley, L. M., *J. Path. and Bact.*, 1943, **55**, 295.

¹⁶ Warren, S., and Olshausen, K. W., *Am. J. Path.*, 1943, **19**, 307.

¹⁷ Ewing, J., *Neoplastic Diseases*, 4th ed., p. 854, 1940.

¹⁸ Innes, J. R. M., *J. Path. and Bact.*, 1942, **54**, 485.

¹⁹ For example: Bonser, G. M., *J. Path. and Bact.*, 1944, **56**, 15; Gardner, W. U., *Cancer Research*, 1943, **3**, 92; Hooker, C. W., and Pfeiffer, C. A., *Cancer Research*, 1942, **2**, 759; Shimkin, M. B., and Andervont, J. B., *J. Nat. Cancer Inst.*, 1942, **2**, 611.

¹² See pl. 5, Baldwin, F. M., and Goldin, H. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 813.

¹³ Baldwin, F. M., and Li, M. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **40**, 601.

no previous descriptions of the testicular tumor in teleost have been noted. Since the tumors were not found in normal untreated fish in our laboratory the question of hereditary predisposition of the neoplasm cannot be answered at the present.

The tumor-producing action of corn oil on tissue has been investigated by Burrows and Johnston.²⁰ Literature reveals that sesame oil may act as a stimulator or inhibitor to certain physiologic activities of tissues or

²⁰ Burrows, M. T., and Johnston, C. G., *Arch. Int. Med.*, 1925, **36**, 293.

²¹ Caspari, W., and Ottensooser, F., *Z. f. Krebsforsch.*, 1930, **32**, 74; 1932, **38**, 351 (Stern, K., and Willheim, R., *The Biochemistry of Malignant Tumors*, p. 406, p. 450, Reference Press, N.Y., 1943.)

organs. Caspari and Ottensooser²¹ claimed that peroral administration of this oil may even impede the growth of transplanted tumors. The chemical nature of sesame oil has been discussed by Bruce and Tobin.⁵ It seems that the actions of this oil might be due to some inclusions in the oil. Further studies now in progress using extracted fractions of this oil should help to settle the problem.

Summary. Interstitial cell tumors of the testicle occurred in 3 of 40 adult male teleost (*Xiphophorus helleri*) that had received retroperitoneal injections of sesame oil. Two of the tumors were malignant and the other was local nodular hyperplasia. Damage to other organs and some other effects of the oil treatment were observed. No neoplastic changes were found in the untreated adult male fishes.

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A Difference in Cardiodecelerator Action Between Digitoxin and Digitoxigenin.*

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The cardioaccelerator action of the cardiac glycosides is not due to a single mechanism. A direct effect upon the mammalian heart, demonstrable in the isolated denervated organ, as well as effects mediated by the nervous system, play a role. One of the established sites of action involving the vagal mechanism is the carotid sinus, as was shown by Heymans and Heymans.¹ A central vagal stimulation was assumed by Cushny.² However, direct experimental evidence for it rests only on

experiments by Greene and Peeler³ on the innervated turtle heart in which the head was included in a perfusion system separate from that of the heart; while in dogs Heymans and Heymans were unable to observe a central vagus stimulating action of strophanthin when this was applied to the central nervous system in cross-circulation experiments.

Recently Walther⁴ has drawn attention to a pharmacological difference between digitoxin and its aglucone, digitoxigenin. In electrocardiographic studies in intact cats, he noticed an immediate decrease in heart rate after the intravenous administration of digitoxigenin. No such change occurred after digitoxin.

Employing the innervated isolated heart of the dog in a cross-circulation method in

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¹ Heymans, J. F., and Heymans, C., *J. Pharm. and Exp. Therap.*, 1926, **29**, 203; *Arch. internat. de Pharmacodyn. et de Thérap.*, 1926, **32**, 9.

² Cushny, A. R., *J. Exp. Med.*, 1897, **2**, 233. For complete literature, see Lendle, L., in Heffter's *Handbuch der Pharmakologie. Ergänzungswerk*, 1935, **1**, 161.

³ Greene, C. W., and Peeler, J. O., *J. Pharm. and Exp. Therap.*, 1915, **7**, 591.

⁴ Walther, R., *Arch. f. exp. Path. u. Pharmacol.*, 1940, **195**, 709; 1943, **201**, 611.