location in the circulation, the spleen can clear the circulating blood from bacteria and other foreign material by its macrophage system. This clearing function of the spleen is limited but is important.

On the other hand, the spleen has been known to be essential for the recruitment of lymphocytes to the antigenic site for sensitization[7] as well as the production of antibody against a particulate antigen[12]. In this study a significantly lower hemolytic antibody production was found in splenectomized rats (table). A defective production of opsonins, leukophilic α-globulin, interferon and antibodies has also been reported in splenectomized men and animals[5,7,14,15].

As a conclusion, this study clearly shows that the spleen, with its dual role as a phagocytic clearing function and an antibody producing organ, may be an important organ in defence against infection.

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Steroid-induced concentric membrane whorls in dog liver

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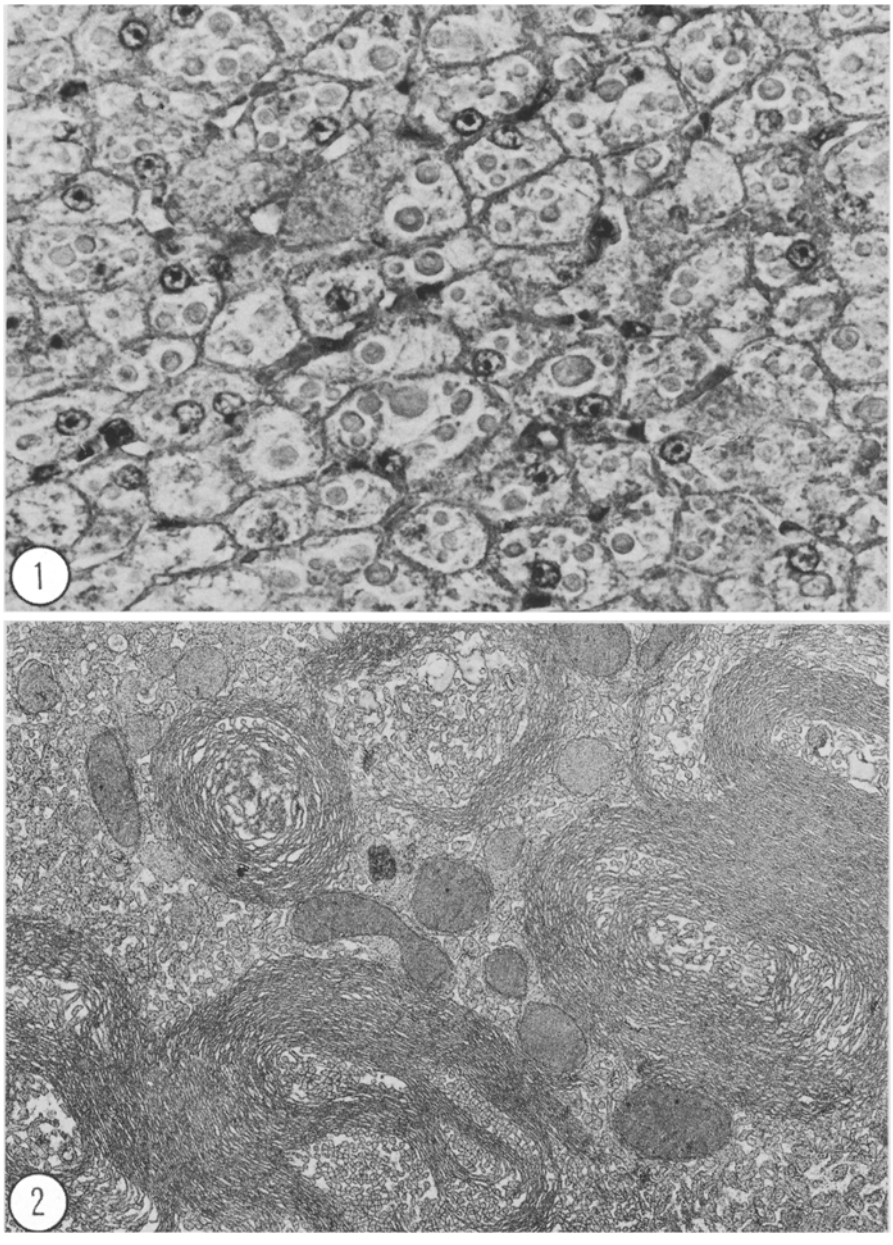
Summary. When daily doses of 10 mg/kg of the androgenic steroids fluoxymesterone, methyltestosterone, testosterone propionate, oxymetholone and mepitiostane were administered to adult male and female beagle dogs for 6 months, concentric membrane whorls were produced in the hepatocytes of all groups. The whorls frequently had a central core mainly composed of lipids or mitochondria and the membranes of the whorls, consisting of paired membranes, continued to the smooth or granular endoplasmic reticulum at the periphery of the structures.

In the course of toxicity studies, we observed that fluoxymesterone (FM), a steroid having androgenic activity, induces intracytoplasmic inclusion bodies of a reversible nature in hepatocytes of beagle dogs after 6 months of oral administration of 20 mg/kg daily. The bodies were round to oval, ranging in size from 1.5 to 15 µm and had a laminated appearance with concentric rings. Inclusions were found mainly in the centrilobular zone and up to 8 per hepatocyte were counted. In paraffin sections, the inclusions could be stained with eosin, chromotrope 2R, methazole fast blue 2G and Sudan black B, but not by the PAS reaction. Electron microscopy revealed that the bodies were composed of paired membranes continuing to the smooth or granular endoplasmic reticulum at the periphery. These membranes were usually arranged concentrically, resembling fingerprint structures[1], and often had a central core composed of lipids, mitochondria and, less frequently, other cytoplasmic organelles. Concentric lamellar formations have been reported in the livers of rats to which various agents have been administered[1-4], but formation by androgenic steroids has not been reported in animals or man. Therefore, we conducted a newly designed study using 5 androgenic steroids: FM, methyltestosterone (MT), testosterone propionate (TP), oxymetholone (OM) and mepitiostane (MTS).

Daily doses of 10 mg/kg of these steroids were administered for 6 months to adult male and female beagle dogs with 6-8 dogs per steroid group. The drugs were administered p.o. using gelatin capsules, except the TP group which was subjected to s.c. injections. An additional 6 control dogs, 3 males and 3 females, received only sesame oil p.o. Plasma levels of cholesterol, phospholipids, triglyceride, GOT, GPT and alkaline phosphatase, and BSP excretion were determined at 1, 3 and 6 months of treatment. At the end of 6 months, the dogs were sacrificed and their livers were immediately removed and weighed. Liver samples were examined with conventional light and electron microscopes.

In hematoxylin-eosin stained sections, intracytoplasmic inclusions were frequently observed in the hepatocytes of all dogs that received FM (figure 1). For OM- or MTS-treated dogs, careful observation disclosed hepatocytes with few bodies at a frequency of less than 1 hepatocyte per 400 high-power field of the centrilobular zones, while the livers of MT- or TP-treated dogs as well as control dogs showed no bodies. On the other hand, the EM readily revealed formations of concentric lamellar structures consisting of the double membranes in all groups except the control group (figure 2). The frequency of appearance was FM > MT > TP > OM ≒ MTS, and there were no remarkable differences in the features of the structures. Additional findings were an increase of smooth endoplasmic reticulum (SER) and microbodies, and a slight deformation of mitochondria. 2 of the 8 animals in the FM group showed evidence of intrahepatic cholestasis, although no necrosis was observed. Other organs showed changes thought to be related to the androgenic activity of the steroids such as atrophy of the testis, adrenal and beta cells of the pituitary, and hypertrophy of the prostatic gland and kidney. It was noteworthy that the prostatic gland was exceptionally atrophied in FM-treated males, and some females treated with the 5 steroids showed bone formation deep in the clitoris. Biochemically, plasma levels of cholesterol, phospholipids, and triglyceride decreased in each steroid-



Photographs show hepatocytes from dog which received 10 mg/kg of fluoxymesterone p.o. for 6 months. Fig. 1. A cluster of intracytoplasmic inclusion bodies with a laminated appearance is seen. H-E, ×623. Fig. 2. Electron microscopically, the bodies are composed of concentrically arranged membranous whorls of paired membranes. (× 10, 400).

treated group by about 1/3 to 1/2 of the control values. Increased plasma GOT and GPT activities, the latter being more pronounced, were found in some dogs of each steroid-treated group, whereas alkaline phosphatase activities and BSP excretions were within normal ranges except in 1 of 2 dogs with intrahepatic cholestasis. These changes did not differ to any considerable extent among the 5 steroid groups and there were no distinct correlations between the severity of these changes and the formation of the concentric lamellar structures. The structures have been regarded as evidence of regeneration[5], degeneration[3], hypertrophy[1] or interference with catabolism of SER by the administered drug[6]. In the present study, hepatocytes of the steroid-treated groups showed increase of SER. The concentric membraneous whorls, which are composed of SER, may provide an increased membrane surface for contact of the steroids with the detoxification enzyme system. Thus, whorls of paired membranes seem to be a structurally modified form of proliferated SER and are one of the adaptative response of the liver to a large dose of weakly toxic foreign steroids.

Recently, we confirmed by means of serial needle biopsy on 2 dogs treated with 60 mg/kg of FM daily for 15 days, that intracytoplasmic inclusion bodies in dog liver are produced within 3 or 6 days of treatment and the bodies are markedly reduced in number and size at 30 days after the cessation of the treatment.

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