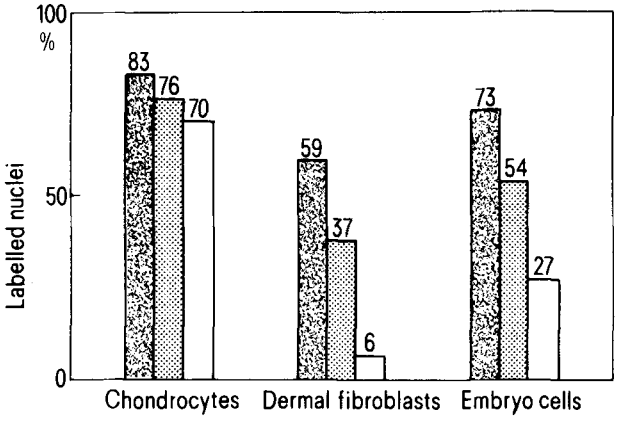
(PDS+PR), and 70% in 5% PDS. Although WBS seemed a little more stimulatory than PDS+PR, or PDS, all 3 types of sera were effective for inducing DNA synthesis of chondrocytes. On the other hand, 59% of the whole nuclei of dermal fibroblasts were labelled in 5% WBS, but only 6% were labelled in 5% PDS. When the PDS was supplemented with the releasate from platelets, the serum (PDS+PR) recovered its growth-promoting activity, although the recovery was not complete, probably because insufficient platelets were added compared with those in normal blood. In 5% PDS the pattern of ^{3}H-thymidine uptake by nuclei of embryo cells was intermediate between those of chondrocytes and dermal fibroblasts. This result may reflect the fact that, although embryo cells are certainly mainly from the mesoderm, they are a mixed population of chondroblastic cells and other fibroblastic cells. In the present experiments, these 3 types of cells did not show 100% DNA synthesis even in 5% WBS. However, it seems reasonable that a certain fraction of the total cells did not divide, because these cells were not established cell lines. The results indicate that the growth factor from platelets is essential for growth of hamster dermal fibroblasts, but not for that of hamster chondrocytes. Thus it is concluded that PGF is not effective as a growth factor for growth of all mesodermal cells, but only for growth of a rather limited portion of mesodermal cells. Although it is well established that chondrocytes grow well in cell culture and that serum is necessary for their growth like that of fibroblasts, another growth factor(s), such as somatomedins[11], may be responsible for their growth. The growth responses of other types of mesodermal cells, such as myoblasts and kidney cells to PGF require further investigation.



Percent of labelled nuclei in hamster chondrocytes, dermal fibroblasts and embryo cells cultured with ^{3}H-thymidine (4 μCi/ml) for 48 h. Closed bars, cultured in 5% WBS; stippled bars, cultured in 5% (PDS+PR); open bars, cultured in 5% PDS.

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The effect of splenectomy on the development of experimental pyelonephritis

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Summary. Although an unusually high incidence of a variety of infections in infants who had undergone splenectomy has been reported by a number of investigators the subject remains somewhat controversial. In the present experiments the role of the spleen in the protection of rats against experimentally induced hematogenous pyelonephritis was studied. The results of this study suggest that the spleen has an important preventive function against bacterial infection.

Splenectomy is performed to treat certain diseases[1,2]. The idea that the spleen is not a physiologically essential organ has been widely accepted since most individuals whose spleen is removed, whatever the reason, do not develop any significant disturbances. But, in 1952 King and Schumaker[3] reported an unusually high incidence of infection in infants who had undergone splenectomy for congenital hemolytic anemia. The important function of the spleen in the prevention of various infections has been reported in a number of papers[4–7] in spite of some conflicting results[8].

It is known that *Staphylococcus aureus*, a most abundant bacterial species, induces many infections in man. As described previously[9–11] we have demonstrated that *Staphylococcus aureus* is one of the most active bacteria in producing experimental haematogenous pyelonephritis in rats. This method, which is simple to perform and easy to quantify, has been successfully applied to the study of the effects of certain antibacterial agents in vivo. The present study was undertaken to investigate whether splenectomy has any effect on the development of experimental haematogenous pyelonephritis in rats.

Materials and methods. 20 young inbred white rats *(Rattus norvegicus* var. *albino)* weighing 75–100 g, obtained from the Experimental Research Center of Istanbul Medical Faculty, were used. The animals were fed on a commercial pellet food for laboratory animals and tap water ad libitum throughout the experiment. At the beginning of the study the rats were divided into 2 groups. 13 rats were splenecto-

Score of pathological findings, the number of viable *S. aureus*/ml of urine and mean serum hemolytic titers are shown. Each value represent mean ± S.E., and figures within parenthesis indicates the number of animals in each group

	Scores of pathomorphological findings	Number of viable *S. aureus* per ml in urine	$\log_2$ of hemolytic titers
Sham-splenectomy	1.85 ± 0.26 (7)	41.57×10^6 (7)	10.42 ± 1.21 (7)
Splenectomy	3.60 ± 0.16 (13)***	157.57×10^6 (7)**	6.42 ± 0.78 (7)*

*$p < 0.02$, **$p < 0.01$ and ***$p < 0.001$, when compared with sham-splenectomized animals (Student's t-test).

mized and 7 rats were sham-splenectomized, under general anesthesia using sodium pentobarbital (50 mg/kg) in aseptic conditions.

45 days later experimental haematogenous pyelonephritis was produced in both groups by injecting 1 ml of a suspension containing 5×10^8 *Staphylococcus aureus* into one of the tail veins. 10 days after the injection blood was aseptically collected by cardiac puncture under i.p. pentobarbital anesthesia. The urinary bladder was exposed through a midline abdominal incision and urine was aspirated by means of a sterile syringe and needle. Pathomorphological findings on the viscera were carefully noted. Macroscopic and microscopic findings were rated from 0 to 4, as seen below: 0, no lesion; 1, only microscopic lesions; 2, formation of 1-2 abscesses macroscopically; 3, several abscesses; 4, widespread abscesses.

In another experiment 7 splenectomized and 7 sham-operated rats were immunized with sheep red blood cells (SRBC). The immunization was performed by injection of approximately 10^8 SRBC into the tail vein. Serum hemolysin titers of both groups were determined 14 days after immunization, using the method described by Sinclair and Elliott[13].

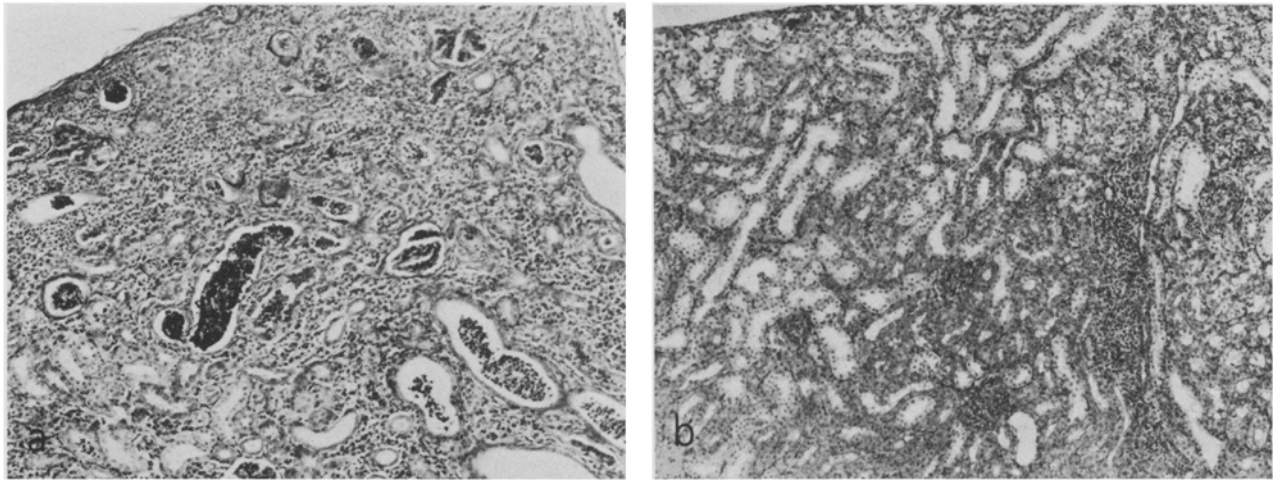
Results. Pathological findings of pyelonephritis were more evident in the splenectomized rats compared to those subjected to sham-splenectomy. As can be seen in the table, the mean scores of sham-splenectomized rats were significantly less than those of the splenectomized rats. Widespread pyogenic abscesses from the cortex to the pelvis of the kidneys were observed in the splenectomized rats. Additional abscess formation in the abdominal viscera and adhesions in between were seen in some rats of this group. In the microscopical examination (figure), this group showed a typical picture of an experimental haematogenous pyelonephritis characterized by abscesses formed by numerous leucocytes and exudation in some enlarged tubular lumina, and some glomerular lesions such as thickening of the parietal layer of Bowman's capsulae and scarring of some of the glomeruli. Qualitatively similar pathomorphological findings were found in sham-splenectomized control rats (figure).

The bacterial culture of blood collected on the 10th day of pyelonephritis from both groups remained sterile. But abundant viable *Staphylococcus aureus* were determined in the urine of the both groups. The mean values for the numbers of viable bacteria in the urine of both groups are shown in the table. As can be seen, the mean values for the splenectomized rats were significantly higher than those for the sham-splenectomized rats.

Serum hemolysin titers of both splenectomized and sham-splenectomized groups were also shown in the table. As can be seen the mean hemolytic antibody titers of splenectomized group were significantly lower than those of the sham-splenectomized group.

Discussion. The results of the present study indicate that splenectomy increased susceptibility to experimental haematogenous pyelonephritis in rats. As evidenced by pathological findings (figure and table) large and widespread abscesses were found in the kidneys, urinary tract and other viscera of splenectomized rats. The pathological findings are significantly less obvious in sham-splenectomized rats. Moreover, the abundant bacteriuria which was observed in splenectomized rats also indicated that there was a severe pyelonephritis in them (table). An increased susceptivility of splenectomized children to various infections has been reported in many investigations[1-3,5]. These findings have been supported in a number of experimental studies[4,5].

The important preventive function of the spleen against infections has been explained on the basis of a dual role of this organ. As reported by Ellis and Smith[5], in its strategic



The light microscopic views of pyogenic abscesses in the kidneys of a splenectomized (a) and a sham-splenectomized rat (b). The procedure for induction of pyelonephritis and description of pathological findings are in the text. × 80.

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-