Inhibition of collagen-induced aggregation (figure 2,D) is also observed in fraction 3 and in all fractions after fraction 6. Anticoagulant activity is found only in fractions 7 and 8 (figure 2,E). This experiment demonstrates the degree of redundancy found for the antithrombotic activity of salivary *R. prolixus* secretion. At least 2 factors inhibit ADP induced platelet aggregation and more than 2 are involved in the inhibition of collagen induced platelet aggregation. The anticoagulant activity and the apyrase activity together cannot completely explain the results shown in figure 1, which are thus to be interpreted as the concerted action of many substances. The possibility that protein bound prostaglandins could be involved was ruled out as the extraction procedure for plasma prostaglandins[11] was performed with *Rhodnius* saliva and the final evaporated ether extract was not an inhibitor of platelet aggregation. Our data indicate that a complex system for inhibiting platelet aggregation is present in the saliva of *Rhodnius prolixus*. This system together with the anticoagulant activity may provide the insect with an antihemostatic machinery important for the completion of the meal in a small lenght of time. To assess how generally such mechanisms are distributed, it would be interesting to study the presence of antithrombotic substances in other hematophagous insects.

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The growth responses of hamster chondrocytes, dermal fibroblasts and embryo cells to whole blood serum and plasma-derived serum[1]

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Summary. Autoradiographic studies with ^{3}H-thymidine demonstrated that the growth responses of hamster chondrocytes, dermal fibroblasts and embryo cells, respectively, differed in media containing whole blood serum (WBS) and plasma-derived serum (PDS). Dermal fibroblasts seemed to require a growth factor from platelets for growth, but chondrocytes did not. Embryo cells showed an intermediate pattern of growth response to this factor.

Recently, several investigators[3-5] have shown that platelets are the source of a growth factor in serum called the platelet-derived growth factor (PGF). Serum from whole blood (whole blood serum (WBS)) contains the factor because it is released from platelets during blood coagulation, whereas serum from plasma (plasma-derived serum (PDS)) lacks the factor because plasma is essentially free of platelets. It has been reported that PDS is unable to stimulate proliferation of several types of cells, such as primate arterial smooth muscle cells and dermal fibroblasts[6], mouse 3T3 cells[4,5], and human glial cells[7]. This paper reports differences in the growth responses of hamster chondrocytes, dermal fibroblasts and embryo cells to WBS and PDS.

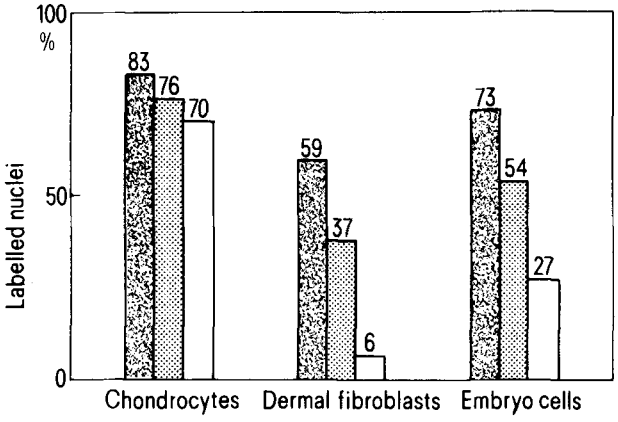
Materials and methods. Chondrocytes were obtained from 1-week-old hamsters as described previously[8]. Dermal fibroblasts were obtained as follows: The skin of the back of 1-week-old hamsters was sterilized with 70% ethanol, separated and rinsed twice in Hanks' solution. The tissue fragments were minced with scissors and trypsinized with 0.25% trypsin. Then the dermal fibroblasts were cultured in Ham F-12 medium supplemented with 10% fetal calf serum. Embryo cells were obtained from embryos on the 13th day of gestation as described previously[9]. Primary cultures of these 3 types of cells were trypsinized and stored in liquid nitrogen for use as standarized cells.

Blood was collected from 8-month-old male SD rats in plastic tubes containing 1/10 vol. of 3.8% sodium citrate. The pooled blood was divided into 2 fractions for preparation of WBS and PDS by the method of Ross et al.[10]. Platelets were obtained from rats, added to the PDS, and recalcified by adding 1M $CaCl_2$ to give a concentration of 20 µmoles/ml. The resulting serum was centrifuged at 22,000 × g for 30 min at 4 °C, and the supernatant was dialyzed against Ringer solution for 24 h at 4 °C, filtered through a Millipore filter, and used as PDS + PR (platelet releasate).

Secondary cultures of the cells were trypsinized and seeded into Lab-Tek tissue culture chambers (4802: Lab Tek Products, Illinois, USA) at a density of 5×10^4 cells/chamber in Ham F-12 medium supplemented with 5% concentrations of the 3 types of serum: WBS, PDS, and PDS + PR. Then ^{3}H-thymidine (4 µCi/ml; 6.7 Ci/mmole: New England Nuclear, Massachusetts, USA) was added to each dish and the cells were incubated for 48 h at 37 °C. After incubation, the cells were fixed with methanol, washed twice with cold 5% trichloroacetic acid and processed for autoradiography. Approximately 1000 cells were counted for each point.

Results and discussion. The results are shown in the figure. As can be seen, 83% of the nuclei of chondorocytes were labelled with ^{3}H-thymidine in 5% WBS, 76% in 5%

(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-