Results. The titres of the sera from the various categories of patient are given in the table

Category	Titre 0	1/2	1/4	1/8	1/16	1/32	1/64	No: elevated (> 1/16)
Normal	46	20	8	4				0
Nephritis	22	5	4	10	0	1	1	2/43
Lipodystrophy	5	4						0
Leukaemia	2	9	1	4	1			1/17
Bowel disease	0	0	8	4	4			4/16
Cirrhosis	6	3	2	2	2			2/15
Pre-transplant	0	6	4	7	3			3/20
Post-transplant	0	24	12	24	21	7	3	31/91
Haemolytic-uraemia	3	3	0	0	1	0	1	2/8
Gram-negative sepsis	0	0	0	1	1	2	0	3/4

Discussion. Since a Re mutant only possesses lipid-A on its cell wall, the antigen is convenient for measuring anti-lipid-A (anti-endotoxin) antibody titres. It is clearly worrying that the normal population and patients with such conditions as leukaemia have only low titres. The rise of titre in transplant recipients may reflect occult urinary tract infection[7] but it does raise the question as to whether cross-reacting antigens involved in rejection can cause a rise of anti-lipid-A titres. Haemolytic-uraemia is known to follow bacillary dysentery[8] and has always been regarded as a Shwartzman equivalent[9]. However these results add support to the view that the pathogenesis is heterogenous[10].

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Participation of granulocytes and humoral factors in resolution of platelet aggregates during endotoxemia

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Summary. Hydrogen peroxide generated by phagocytizing granulocytes can prevent platelet aggregation induced by ADP or collagen but not by endotoxin. Endotoxin tolerance enhances granulocyte mobilization in response to endotoxin and reduces aggregation induced by endotoxin but not ADP or collagen.

Persistence of endotoxin in the bloodstream has been associated with the damaging effects of the toxin[1-5]. Platelets are important in clearance of endotoxin[6] and other particulate matter from the blood[2,7,8], but passage of platelet-particle complexes through the capillary network of nonreticuloendothelial (RE) organs to reach RE tissue depends on maintenance of a balance between aggregation and disaggregation of platelets[8].

Recently we reported that a humoral factor from rabbits made tolerant to the lethal effects of endotoxin makes platelets resistant to the irreversible aggregation normally induced by the toxin[9]. Tolerant animals are also known to mobilize granulocytes more effectively than normal animals[10]. These cells may work with platelets in the delivery of the toxin to the RE system. Granulocytes are attracted to platelet thrombi and participate in their subsequent resolution through phagocytosis[11,12] and release of mediators[13-16]. Hydrogen peroxide (H_2O_2) is a leukocytic mediator released during phagocytosis[17,18] which regulates aggregation induced by ADP and other nonmicrobial substances[14-16]. Presently it is not known whether H_2O_2 attenuates endotoxin-induced aggregation or whether endotoxin tolerance alters reactivity to aggregating agents other than endotoxin. The following studies were undertaken to resolve these questions.

Platelet-rich plasma (PRP) was obtained from heparinized blood taken by cardiac puncture from adult New Zealand white rabbits[9]. PRP was reacted with ADP (Sigma), calf skin collagen (Sigma), or *Salmonella typhi* endotoxin (Difco) in plastic cuvettes in a spectrophotometer adapted to measure aggregation[9]. In some cases platelets were treated with 3 µl (4.5 µm) of 3% H_2O_2 (Baker) approximately 100 sec before addition of the aggregating agent. We had previously observed that this concentration of H_2O_2 provided maximum effects without inducing aggregation or optical interference.

In the first set of experiments, normal PRP was diluted 1:1 with cell-free plasma from either tolerant or normal rabbits. Plasma from tolerant rabbits causes normal cells to respond to endotoxin more rapidly and reversibly, as previously observed[9]. In contrast, platelet responses to collagen (25 µl of stock solution containing 7.8 mg per ml of pH 7.4 Tris buffer) or ADP (2 µM) were unaltered by this treatment. Dilution of PRP with plasma from normal rabbits did not affect aggregation patterns.

Tolerant rabbits challenged i.v. with 50 µg of endotoxin

were able to mobilize leukocytes more effectively than normal animals (figure 1). Phagocytosis of platelet aggregates by these cells is paralleled by H_2O_2 formation and release[17,18]. Therefore effects of this substance on platelet aggregation induced by ADP, collagen, or endotoxin were determined (figure 2). H_2O_2, as indicated by others[19], reduced aggregation induced by ADP or collagen. However, H_2O_2 was ineffective against endotoxin-induced aggregation.

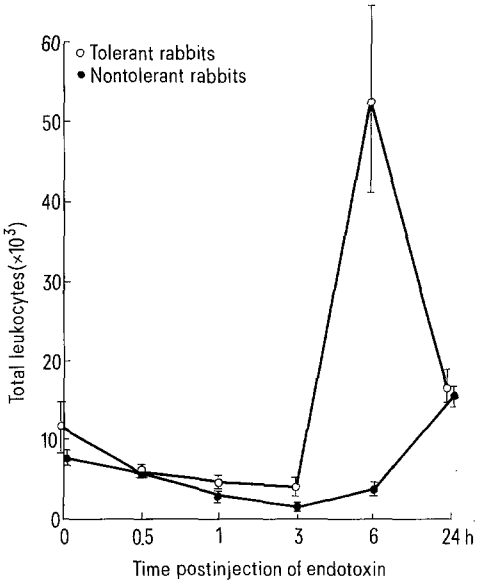

Fig. 1. Alterations in total circulating leukocyte numbers in tolerant and nontolerant rabbits challenged i.v. with 50 µg of *S. typhosa* endotoxin. 3–5 animals were used to make each determination.

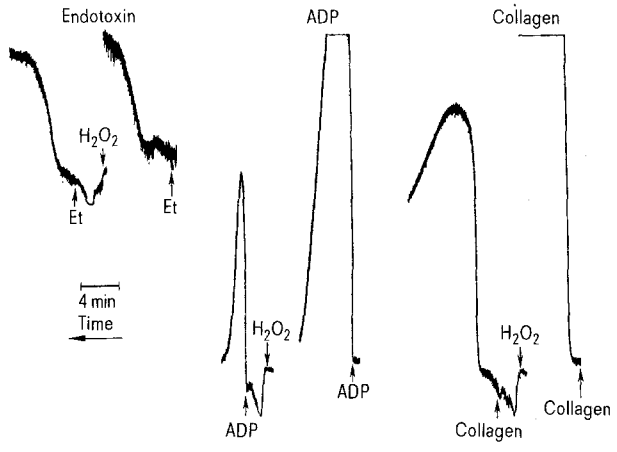

Fig. 2. Effect of treatment of normal rabbit platelets with H_2O_2 on aggregation induced by endotoxin, ADP, or collagen.

In summary, we found that one major leukocytic regulator of aggregation, H_2O_2, does not alter endotoxin-induced platelet aggregation. Elaboration of H_2O_2 during phagocytosis may limit additional aggregation after endotoxin is trapped, thereby reducing the phagocytic load of the leukocytes and the subsequent release of their mediators, which can contribute to the lethal consequences associated with the toxin[20–22]. Altered platelet aggregation characteristics and rapid leukocyte mobilization associated with tolerance could enhance clearance of platelet-associated endotoxin from the microcirculation and its transport to the RE system for further processing.

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Effect of temperature and light on the production of androgens in the male *Rana esculenta*[1]

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Summary. The present data show that experimentally-controlled environmental variables (light and temperature) can alter circulating androgen levels in the male green frog, *Rana esculenta*, treated in different phases of the testicular cycle.

Rana esculenta has a potentially continuous type of spermatogenesis. Histological studies suggest that high temperature stimulates pituitary gonadotropin secretion and spermatogenesis, whereas the reverse occurs at very low temperatures[2]. These results also suggest that various aspects of the testicular cycle show a differential sensitivity to environmental cues. The plasma levels of testosterone are high in late winter and very low in summer[3]. Thus, although the