blastocysts obtained from conditions which are more conducive (+ progesterone) vs less conducive (no hormone) for viability maintenance can be compared. To this end, days 19–23 blastocysts from ovariectomized mothers either receiving progesterone or no hormone were labeled for 4.8 h, a period intermediary to 3 and 6 h when incorporation appears to be linear with time. As predicted, the amount of radioactivity found in the group with fewer optimally viable embryos (no progesterone) was elevated in comparison to the group treated with progesterone (table, B). Thus, the hormonal state of the mother at the time of sacrifice or following ovariectomy appears to be functionally related to the diminished incorporation activity of diapausing blastocysts rather than their gestational age. The lower incorporation values found through day 9 p.c. but not after day 12 p.c. would appear to be the result of prolonged biological activity of progesterone on uterine tissue or its fluid possibly in conjunction with sustained slow depletion of hormone from fatty tissue and serum protein complexes[26]. Since diapausing blastocysts become less viable after day 15 p.c. in the absence of progesterone[4,27], the increased incorporation observed after prolonged existence in utero in the absence of progesterone supplements may indicate deterioration of the embryo rather than physiologically normal 'activation' of late diapausing embryos.

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Anti-endotoxin (anti-lipid-A) antibodies

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Summary. Anti-lipid-A, anti-endotoxin, antibodies have been measured by a passive haemolysis test using antigen from an *E. coli* Re mutant. Titres in the normal population are low but do rise in situations in which there has been gram-negative sepsis. Absence of raised titres in other situations has profound implications.

The endotoxin of gram-negative organisms consists of a distal O-polysaccharide antigen, a central core R-antigen, and then a toxic lipid-A moiety that is attached proximally to the bacterial cell envelope. In this position the toxic lipid-A could be a concealed antigen as it is obscured by the overlying hydrophilic polysaccharide chains that tend to form a shielding gel. Lack of antibodies in the normal population would explain why the effects of endotoxins are so devastating[2,3]. There is indeed evidence that only persons who have previously suffered gram-negative sepsis or who have chronic pyelonephritis or inflammatory bowel disease do have raised anti-lipid-A antibody titres[4–6]. In order to assess the position we have measured anti-lipid-A antibody titres in selected disease antities.

Method. Sera were inactivated at 56 °C for 30 min and were then adsorbed with washed sheep erythrocytes. 0.2 ml serial dilutions of the sera were made using phosphate buffered saline containing 1% albumin as diluent in U-shaped cups. 0.1 ml of 1/40 guinea-pig complement was added to each well, and then 0.05 ml of a 1% suspension of sheep erythrocytes coated with the lipid-A antigen. This was alkali treated lipid-A of an *E. Coli* Re mutant kindly supplied by Dr Günter Schmidt of the Max-Planck-Institut Freiburg.

The validity of the assay was established by the finding of rising titres 1/8–1/32 in 3 patients who had suffered a gram-negative septicaemia. A titre of 1/16 has been taken to indicate an elevated antibody titre. 2 children who had haemolyticuraemia syndrome in London had raised anti-lipid-A titres after their illness, whilst 6 from Holland did not, even though they were uraemic and jaundiced. Patients with inflammatory bowel disease had moderately elevated titres but such patients were surprisingly few among patients with primary biliary cirrhosis or early alcoholic cirrhosis and among patients with leukaemia.

Of particular note are the results of the anti-lipid-A titres performed serially on renal transplant recipients. All showed a rise in titre in the 2nd or 3rd week post-transplant to 1/16 or 1/32. Only one had a definite urinary tract infection, 3 were considered to have rejection and another a chest infection.

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