

## Morphological studies of cytotoxic lesions in reversible endotoxic shock

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**Summary.** Reversible endotoxic shock was induced in adult rats by intravenous injection of *E. coli* 0111:B4 lipopolysaccharide (LPS) and the progression of metabolic and morphological alterations was evaluated. Serum samples and biopsies from adrenal gland, liver and lung were studied at different times after LPS injection.

Histological changes in these tissues were observed after endotoxin administration, coinciding with both the acute-phase and the recovery-phase of shock (24-72h after LPS injection). Signs of tissue regeneration can be correlated with the regression of some serum parameters to their normal values.

All these results indicate that in this experimental model of endotoxic shock, a reversible status was established, which will allow further studies of the endotoxic pathophysiological mechanisms *in vivo*, avoiding the complexity of the non-reversible process.

**Key words:** Endotoxic reversible shock, Experimental model, Adrenal glands, Liver, Lung

### Introduction

Endotoxic shock is a pathophysiologic phenomenon induced either by the release of Gram-negative bacterial endotoxins (lipopolysaccharides, LPS) (Berry, 1977) or following the experimental exogenous administration of endotoxins (Weil et al., 1956).

Endotoxic shock is one of the most difficult types of shock to treat (Shumer, 1979) and one of the most important clinical problems due to its very high mortality rate.

The sequence of events leading to septic shock begins with a bacterial invasion secondary to a major septic

process (pneumonitis, subacute bacterial endocarditis, peritonitis, etc). The precipitating agents, mainly Gram-negative bacteria, are phagocytosed releasing into the bloodstream either exotoxin or endotoxin (Schumer et al., 1979).

Endotoxins are potent biological components of the outer membrane of Gram-negative bacteria and are mainly composed of lipopolysaccharides (LPS) and proteins. The toxic properties of endotoxins reside in the LPS and most of them are elicited by the lipid A moiety of the molecule (Galanos et al., 1977).

Severe endotoxemia is accompanied by extended serum parameter alterations (Cowley et al., 1969; Hinshaw et al., 1977; Traber, 1985) and structural damage in various internal target organs, such as adrenal glands, liver (MacGovern, 1971) and lung (Clowes et al., 1974). The concept of organ interaction in the development of the septic syndrome is critical to the understanding of this process. It remains to be demonstrated whether sepsis or other stimuli are factors involved in the development of the multiple organ failure syndrome.

Many theories such as complement activation (Aasen et al., 1978), release of vasoactive agents (Altura and Halevy, 1977), coagulation abnormality (Hardaway and MacKay, 1959; Morrison and Ulevitch, 1978) direct cell injury (Pagani et al., 1987), and depletion of RES (Freudenberg et al., 1985) have been implicated in the mechanism leading to endotoxic shock. Reduction in tissue perfusion and oxygenation, secondary to a drop in systemic blood pressure, is characteristic of the shock process (Weil, 1977) in which an irrevocable cycle of events can cause organ, tissue, and cell failure and eventually death.

There is a need for well controlled animal studies in this form of shock because unfortunately, no good animal model of the septic metabolic failure syndrome exists (Siegel et al., 1982).

In an attempt to establish a valid experimental endotoxic model for *in vivo* studies, we have investigated the effect of endotoxin administration on serum parameters (glucose, GOT and albumin) and the

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cytotoxic lesions in target organs (adrenal glands, liver and lung) of rats, under conditions of reversible shock.

## Materials and methods

### Materials

Lipopolysaccharide (LPS) from *E. coli* 0111:B4, obtained according to the method of Whestphal et al. (1952), was supplied by Difco (Detroit, Michigan, USA). The  $A_{260}/A_{240}$  ratio was determined for purity verification (Romanowska, 1970).

Monotest GOT opt. and Monotest Gluco-quant were from Boehringer-Mannheim Diagnostica (FRG). Other chemicals were purchased from Merck (Darmstadt, FRG).

### Endotoxin administration

Male Wistar rats weighing 200-250 g were used in the present study. Standard diet and water were given *ad libitum*. *E. coli* 0111:B4 LPS (1.6 mg/100 g body weight) was administered intravenously (i.v.) in saline (1.6 mg/0.2 ml) and rats were killed after different times according to the experiment. Control animals received equivolume injections of saline.

Blood samples were taken by cardiac puncture. Serum was separated by centrifugation at 680g and 4° C for 15 min and stored at -20° C.

### Analytical parameters in serum

Aspartate aminotransferase (GOT) activity and glucose levels were measured in serum following the manufacturers' recommendations.

Albumin levels in serum were measured by immunoelectrophoresis (Laurell, 1966) modified by Grieninger et al. (1979). Rat albumin and antialbumin serum were gifts from Dr. Lampreave (University of Zaragoza, Spain).

### Optic and electronic microscopy

Organ histology was performed in standard conditions after 3% glutaraldehyde fixation. For routine light microscopy (LM), thick sections, lightly stained with 1% toluidine blue, were examined in a Nikon microscope. For electron microscopy, samples were post-fixed in buffered osmic acid, dehydrated and embedded in Araldite. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined in an Elmiskop 101 (Siemens).

### Statistics

Each experimental point represents the mean  $\pm$  SD of triplicate determinations.

## Results

In order to establish the reversible endotoxic shock 1.6 mg of *Escherichia coli* 0111:B4 endotoxin, per 100 g of body weight, were injected i.v. into rats. The induced endotoxicosis was accompanied by a progressive decrease in rat body weight that can reach 10-20% at 24 h post-endotoxin injection.

To evaluate the reversibility of the shock damage, time-courses of glucose and albumin levels as well as aspartate aminotransferase (GOT) activity, were determined in serum samples obtained at different times after LPS injection.

As is shown in Fig. 1, injection of endotoxin induced a rapid and transient increase in serum glucose levels, which was reverted to hypoglycemia at 2h. This hypoglycemia was more pronounced 6h after *E. coli* endotoxin injection and progressively returned to normal levels.

Aspartate aminotransferase (GOT) activity showed a significant increase (about 100%) 2h after endotoxin injection, reaching the maximum values at 6h (200%). Thereafter, GOT levels decreased and became indistinguishable from the control levels at 72h.

Albumin levels decreased progressively in the sera of LPS-treated animals during, at least, 72h after intoxication, thus indicating a shock process.

Macroscopically the main alteration was observed in adrenal glands, whose weight progressively increased from 6 to 24h after endotoxin injection. The adrenal weight/body weight ratio was 1.5-2-fold higher than that of control values at 24h; this ratio decreased at 72h although it remained above baseline values. Extravasation of red cells and congestion were observed in all tissues, specially in liver and lung.

Histological changes in adrenal glands, liver and lung

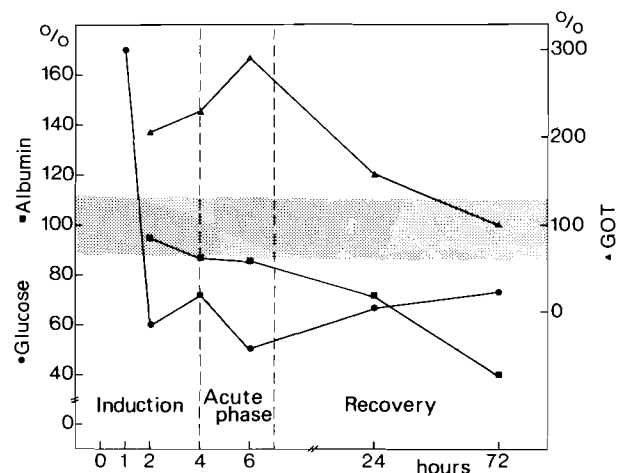
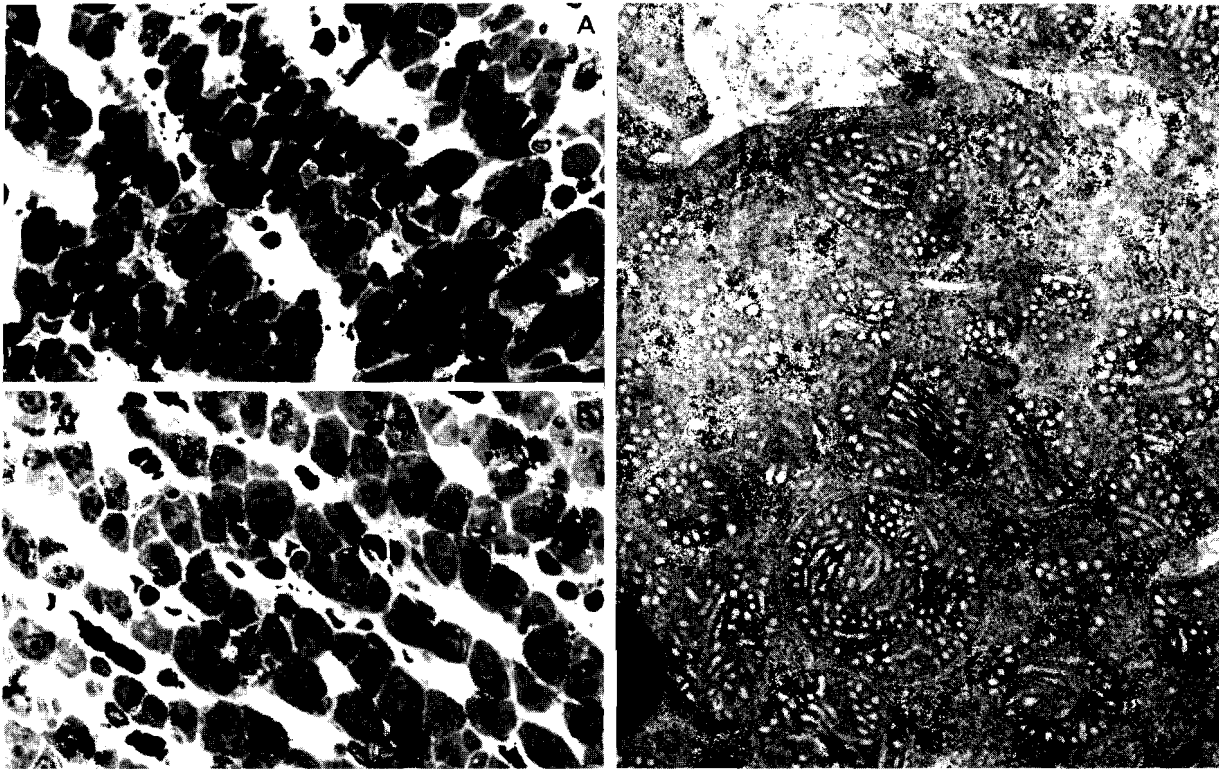
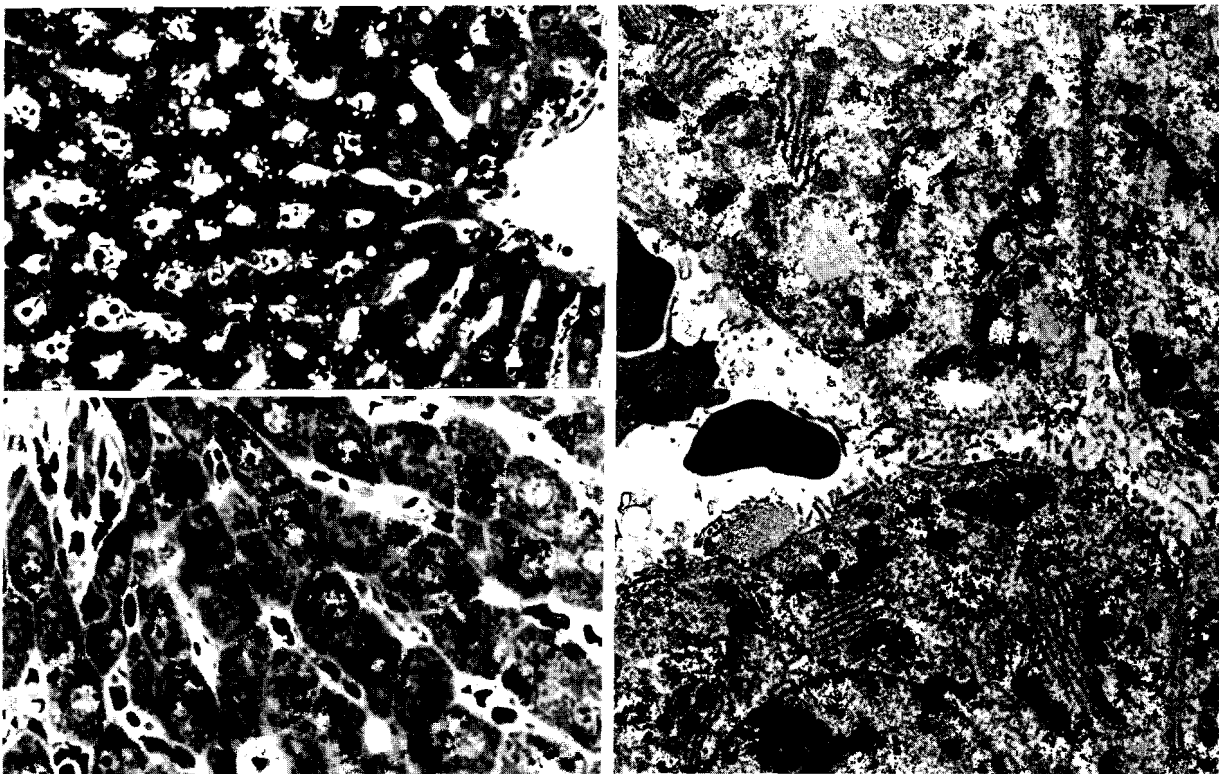


Fig. 1. Time-course of serum parameters during the *in vivo* reversible endotoxic shock.



**Fig. 2.** Micrographs of adrenal gland after reversible endotoxic shock induction. Light microscopical appearance after LPS treatment: A- after 4h.  $\times 100$ , B- after 72 h  $\times 100$  and C- Electron microscopy after 24 h.  $\times 20,000$



**Fig. 3.** Micrographs of liver after reversible endotoxic shock induction. Light microscopical aspect after LPS treatment: A- After 4 h.  $\times 100$ , B- after 6 h.  $\times 200$  and C- Electron microscopy after 6 h.  $\times 8,000$

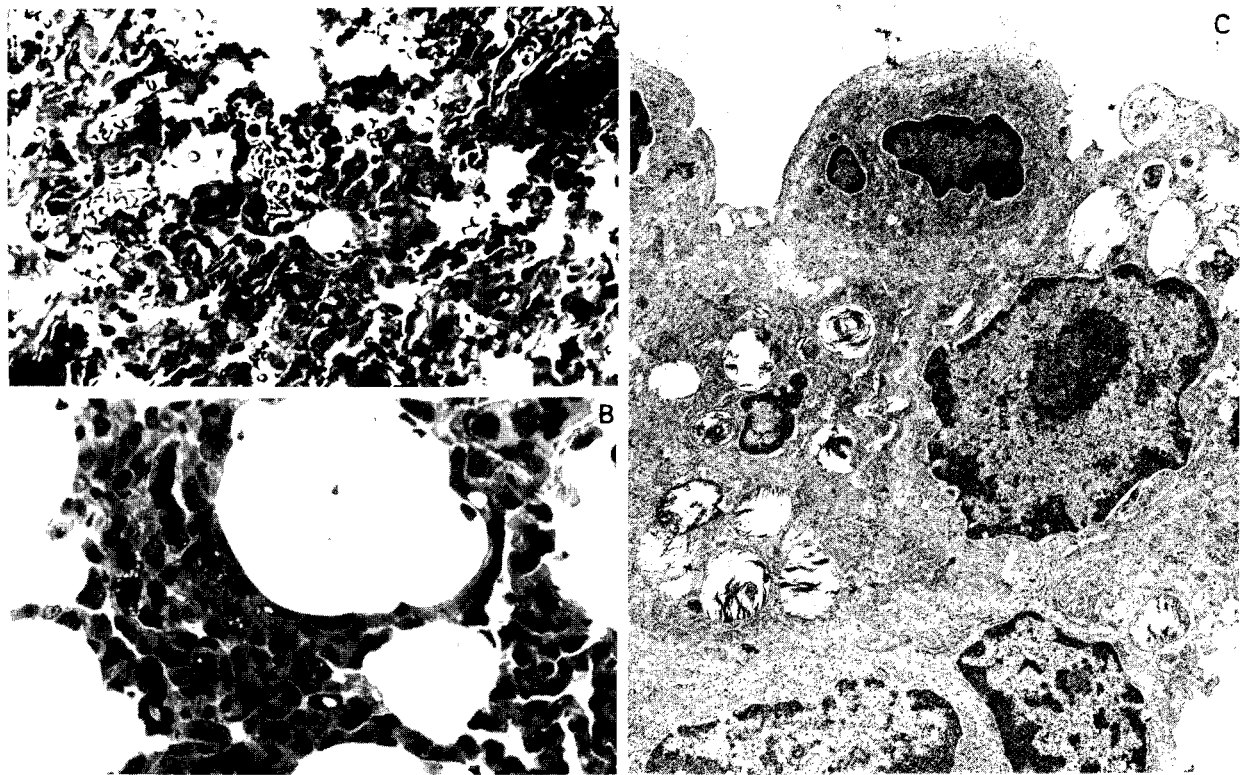


Fig. 4. Micrographs of lung after reversible endotoxic shock induction. Light microscopical aspect after LPS treatment: A— after 4 h.  $\times 100$ , B— after 24 h.  $\times 200$  and C— Electron microscopy after 72 h.  $\times 6,000$

could be observed as early as 4 h after endotoxin administration.

Adrenal glands showed interstitial edema, a slight enlargement of smooth endoplasmic reticulum (4-24 h) (Figs. 2A, 2C) and a decrease in normal cytoplasmic lipid droplets in zona fasciculata cells.

The most affected organ was the liver which exhibited disseminated intravascular coagulation and cellular degeneration around central veins (Fig. 3A), whereas the periportal areas remained normal. At 6h (acute phase) the affected zones also showed a significant increase in rough endoplasmic reticulum (Figs. 3B, 3C).

In lung, the main changes were extravasation of red cells, interstitial edema, leukocytosis (Fig. 4A) and a septum increase, these alterations being more evident 24 h after endotoxin injection (Fig. 4B).

Evidence of tissue regeneration was seen in adrenal glands, and lung 72 h after endotoxin injection (Figs. 2B, 4C).

## Discussion

Although the molecular bases for the toxicity induced by Gram-negative bacterial endotoxins are poorly understood, cytotoxic lesions as well as functional and metabolic disturbances occur in different organs.

Patients show significant hemodynamic, hematological and coagulation alterations as well as changes in some biochemical parameters (electrolytes and non electrolytes, blood gases, metabolites and enzymes).

Kidneys, lungs, liver and heart are the main organs affected during the shock process. If the affected organ system is properly supported, complete recovery is possible. However, if perfusion deficits are prolonged, before being corrected, severe organ failures may subsequently result.

In an attempt to establish a useful model of *in vivo* reversible shock we have used Wistar rats, a species which appears to be highly resistant to endotoxic shock.

At the dosis of LPS employed in this model a reversible shock is induced, as evidenced by the time-course of glucose, GOT and albumin levels after endotoxin injection (Fig. 1); 95% of the treated animals showed the described changes during the acute (2-4 h) and the recovery phases of shock. These results are consistent with the response to sepsis observed in humans.

In 300 consecutive shock patients, serum enzyme levels of aspartate aminotransferase (GOT: glutamate oxaloacetate transaminase), alanine aminotransferase (GPT: glutamate pyruvate transaminase), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH), revealed that the mean serum levels of each of these enzymes were higher in the dying patients as compared with survivors (Cowley et al., 1969). The raised levels of enzymes found in serum were the result of their release from damaged cells into the extracellular fluids.

Clinical manifestations of organ injury did not appear early in the onset of shock, although tissue damage could be documented at the cellular level. It was not until late

in the recovery period that the physiopathological changes became clinically apparent.

The morphological alterations observed in adrenal glands, liver and lung (Figs. 2-4) coincided with those shown at the early and the recovery phases of endotoxic shock in patients, which validates the use of LPS as an inducer of a reversible shock-status.

Adrenal ultrastructural alterations, i.e. interstitial edema, enlargement of smooth endoplasmic reticulum and a decrease in cytoplasmic lipid droplets in cells of zona fasciculata (Figs. 2A, 2C), could be well correlated with pathophysiological alterations. Thus, the increased corticosteroid production during the shock course can be related to the enlargement of smooth endoplasmic reticulum, and the depletion of lipid droplets to the decrease in steroidogenesis observed at the recovery-phase (unpublished data). Other authors have also reported hemorrhagic necrosis with extravasation of red cells, massive hematomas, microthrombi, platelets aggregation at various degrees of severity as well as a lipid depletion in zona glomerulosa and fasciculata of the adrenal cortex of animals and humans during endotoxic shock (Hinshaw et al., 1985; Freudenberg et al., 1985).

Liver histology showed disseminated intravascular coagulation and cellular degeneration around central veins (Fig. 3A), although the periportal areas were normal. The affected zones also exhibited a significant increase in rough endoplasmic reticulum (Figs. 3B, 3C) during the acute-phase of shock which can be justified by the high rate of acute-phase protein synthesis during this step.

In studies of patients conducted between 2 and 24h following the onset of shock, light microscopy of the liver revealed a consistent increase in intracellular lipid and a short number of acute inflammatory cells. A focal necrosis was observed in those patients who subsequently exhibited a severe hepatic dysfunction. Dilatation and congestion of the sinusoids and hepatic veins were the most striking histological changes observed. Dilatation of the sinusoids was most marked in the area of the central zone and was coupled with narrowing of liver cords. In some instances, the sinusoidal dilatations nearly reached the portal zone, although in the majority of cases the periportal areas were normal. The affected zones also showed a significant increase in reticulum (Birgens, 1978) corresponding to the congestive and necrotic changes.

Morphological alterations in lung including extravasation of red cells, interstitial edema, leukocytosis and a septum increase (Figs. 4A, 4B) were correlated with the metabolic and membrane permeability changes leading to a «lung shock» state.

Pulmonary failure occurs in man more commonly under sepsis and severe nonthoracic trauma than in many other illnesses (Moore et al., 1969). MacGovern (1982) found lesions (thrombi, alveolar hemorrhage, and hyaline membranes) attributable to septic shock in the lungs of two-thirds of all patients dying of Gram-negative sepsis. A common abnormality of the lung with endotoxemia is pulmonary edema, which appears localized in the

interstitium and later on extends to alveolar spaces. Pulmonary edema interferes with oxygen transfer owing to maldistribution of ventilation, alveolar collapse, and thickening of the alveolar-capillary wall.

The alterations observed in the experimental model described here indicate the establishment of a reversible endotoxic shock that allows the evaluation of metabolic disorders due to the effect of LPS itself, excluding those secondary alterations produced by the general failure of metabolic processes in the terminal phase of shock.

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