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The Effect of Glutathione on Survival of Endotoxin Treated Mice (33470)

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The nonprotein sulfhydryl (NPSH) level in the liver of mice administered endotoxin has been shown to decrease by nearly 25% within 2 hr and to fall to about 45% of normal in 18 hr (1, 2). A similar rapid decrease in the liver NPSH has been reported following a number of other stress conditions, such as, scalding or ligature of hind legs (2). However, stress induced by single or repeated doses of ACTH, or prolonged administration of deoxycorticosterone failed to decrease the NPSH (3). In the liver, glutathione (GSH) has been reported (4) to comprise at least 90% of the NPSH. The role of the NPSH, and therefore the GSH, in tissues has not been clearly established but it is known to be a cofactor for enzymes such as phosphoglyceraldehyde dehydrogenase (5) and a substrate in the detoxification of halogenated aromatic compounds (6, 7). Because of the importance of the known functions of GSH and the unexplained, rapid decrease of this substance following endotoxin treatment, we determined the effect of exogenous glutathione, given before or after endotoxin, on the development of endotoxin shock in mice.

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Male, ICR mice, weighing 20–25 g, were obtained from Rawley Farms (Plymouth, Michigan) and held in the laboratory for at least 1 week before use. Water and food were freely available at all times. The lyophilized endotoxin prepared from *Salmonella enteritidis* by the method of Ribi *et al* (8) was rehydrated in nonpyrogenic saline (Sherman Labs., Detroit) for 3 hr before use. The test mice were injected intraperitoneally with one LD_{50} dose (225 μg) of the endotoxin in 0.2 ml.

The GSH (Sodium glutathione, Schwarz BioResearch, Inc. or Glutathione, Sigma Chemical Co.) was dissolved in pyrogen-free saline at 150 mg/ml. The GSH was administered in a single dose of 0.2 ml at either 1, 2, or 4 hr before the endotoxin, or the endotoxin was given first and those animals received six doses of GSH, three doses at 3-hr intervals beginning at 18 hr and again at 42 hr after the endotoxin. Groups of endotoxin treated animals injected in the same ways with nonpyrogenic saline served as controls for injection trauma.

The administration of exogenous GSH results in an unphysiologic situation, because this compound rarely occurs extracellularly, especially in the blood where it is a com-

ponent of erythrocytes (9, 10). Also, 30 mg of the peptide injected intravenously as the free acid, which was about pH 3.0, usually caused death of the mice within a few minutes after injection. This effect was alleviated by the use of the sodium salt of glutathione or a solution neutralized by sodium bicarbonate, but some delayed deaths were noted. The GSH was better tolerated when given by the intraperitoneal route than when given intravenously. Both 30-/and 60-mg doses of free GSH could be administered intraperitoneally with only occasional deaths, but a 90-mg dose usually resulted in some deaths. The toxicity of neutralized GSH given intravenously may be due to the development of hypotension which was observed (11) in humans to follow injection with this substance. Clark and Geissman (12) reported that, *in vitro*, relaxation of smooth muscle by adrenaline was potentiated by the addition of GSH. In contrast, Hořejsí and Stěba (13) found that the inclusion of GSH in the fluids administered to dogs in experimental hemorrhagic shock resulted in a higher percentage of recovery. The dose rate of GSH used in that study was much smaller and also more dilute than in this study.

Glutathione failed to provide appreciable protection to animals suffering from endotoxin shock when given in a series of six, 30-mg intravenous doses. The intraperitoneal route was somewhat effective, supporting a 17% increase in survival of the mice treated in this way (Table I). Saline administered in the same ways, however, was more effective than the comparable method with GSH. In either case, intraperitoneal administration resulted in greater survival than occurred with intravenous administration.

TABLE I. The Effect on the Survival Rate (at 72 hr) of Mice Injected with Glutathione (30 mg) or Saline at 18, 21, 24, 42, 45, and 48 hr after Endotoxin.

Treatment	% Survivors
Endotoxin control	44.4[a]
Glutathione (i.v.)	44.4
Saline (i.v.)	52.7
Glutathione (i.p.)	61.1
Saline (i.p.)	75.0

[a] The average of four determinations.

TABLE II. The Effect of Pretreatment with a Single Dose of Glutathione (30 mg) on the Response of Mice to Endotoxin.

Treatment (hr)	Route of injection	% Survivors
Control		46.4[a]
Glutathione		
1	i.v.	33.3
	i.p.	50.0
2	i.v.	91.6
	i.p.	83.3
4	i.v.	83.3
	i.p.	91.6

[a] The average of two determinations.

Preliminary studies of mice pretreated with GSH indicated appreciable protection developed when this compound was given either 2 or 4 hr prior to endotoxin. These results were similar in animals administered the GSH either intraperitoneally or intravenously (Table II). Administration of the GSH only 1 hr before the endotoxin failed to protect the animals. In animals injected intraperitoneally with 30 mg of GSH 4 hr before the endotoxin the survival rate, in a series of seven observations, was 63 ± 30 (SD)% against 40 ± 27% for the endotoxin controls. Despite the large standard deviations of these results, the GSH does exert a protective effect. In each of the seven experiments, the intoxicated control group had fewer survivors, and the chi square test applied to the results accumulated from these experiments showed significant protection ($p<0.05$) resulted from pretreatment with GSH.

Conclusion. Injection of mice with 30 mg of GSH, especially intravenously, was complicated by the unphysiologic, extracellular distribution of the exogenous glutathione. The GSH given to mice to replace nonprotein sulfhydryl lost as a result of endotoxin shock provided only slight protection. Survival was significantly improved by intraperitoneal injection of 30 mg of GSH 4 hr before the endotoxin. Thus, there appears to be little therapeutic potential for GSH in endotoxin shock.

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Freezing Human Peripheral Lymphocytes and a Technique for Culture in Monolayers (33471)

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Peripheral lymphocytes grown in culture undergo a blast-like transformation in the presence of specific antigens and substances such as phytohemagglutinin (PHA) and poke weed mitogen (1). Increasing understanding of the mechanism of this phenomenon is leading to new insights concerning the immune response and delayed hypersensitivity (2). Clinical and epidemiologic applications have been limited, however, because techniques are difficult and cumbersome (3).

Our laboratory has been involved in a series of experiments to facilitate the handling and culturing of lymphocytes. We present a relatively simple and inexpensive method for freezing lymphocytes to be cultured at a later date and for growing lymphocytes (which ordinarily attach poorly to smooth surfaces) in adherent monolayer cultures. By freezing lymphocytes we have greater flexibility in timing experiments, in repeating results, and most important in storing white blood cells from patients with rare and fatal diseases. The method of culturing cells in an adherent monolayer enables us to change fluids in the culture without centrifugation, to observe the cells in growth, and to harvest directly on the petri plates or cover slips minimizing manipulation of the fragile lymphocytes and eliminating the technical difficulties of preparation of slides with even distribution of cells.

In the experiments described below we used PHA, PPD, and vaccinia and measured stimulation by autoradiography employing tritiated thymidine.

Methods and Results. General. Peripheral blood was collected for all experiments in plastic disposable syringes which were prewet with 0.5 to 1.0 ml of sodium heparin. The syringe was placed upright from 60 to 90 min at 37° to permit the red cells to settle and the white cell-rich plasma was removed by the method of Mellman (4). The cell-rich plasma was then spun for 15 min at 1000 rpm and the supernatant plasma was removed and stored. The cells were resuspended in media and counted.