PATHOGENESIS OF EXPERIMENTAL SHOCK

IV. Studies on Lysosomes in Normal and Tolerant Animals Subjected to Lethal Trauma and Endotoxemia*

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Little is known of the basic cellular and chemical events responsible for the progressive deterioration of the circulation following severe and protracted shock. Recently, attention has been focused by de Duve (1) on a class of subcellular particles, the lysosomes, in relation to tissue injury. Recent investigations by two of the present authors (2, 3), appear to lend further support to this concept. Furthermore, the observation that challenge with bacterial endotoxin leads to alterations in lysosomes consonant with their proposed function in tissue injury (see accompanying paper) (4) led us to investigate the possible participation of these particles in the generalized deterioration of cell function which occurs during different forms of fatal shock.

Lysosomes possess a variety of hydrolytic enzymes, the activity of which is repressed, under normal circumstances, by a physical separation of enzymes from their cytoplasmic substrates. de Duve has suggested that this repression is based on the presence of a membrane surrounding the particle (1). Disruption of this membrane, as a consequence of cellular anoxia or some other abnormal event, is presumed to liberate the hydrolases and thereby permit them to act upon cellular protein, nucleic acid, and polysaccharides. The key event in the cell autolysis accompanying pathological changes in tissues would therefore be an increase in the permeability of lysosomes. For example, liver injury, as a consequence of hepatic artery ligation, is associated with a large increase in the unbound, active fraction of several lysosomal enzymes (5). To account for the action of ischemia upon hepatic lysosomes, it was proposed that hypoxia, especially the local acidosis which accompanies oxygen lack, is

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responsible for the solubilization and activation of lysosomal enzymes. This was substantiated in experiments in which lysosome-rich fractions of liver homogenates were incubated at acid pH. The release of free enzymes from these particles was greatly accelerated under such circumstances (6). Moreover, the digestive activity of these hydrolases was found to be optimal at an acid pH (1), again a condition favored by tissue acidosis.

In view of the above observations it is not unreasonable to assume that alteration of lysosomal membranes leading to the release of free, active enzymes may occur in the liver and other visceral organs of shocked animals as a consequence of the hypoxia and acidosis which develop in these tissues. It can readily be appreciated that such a mechanism would markedly exacerbate the tissue injury produced in shocked animals and contribute to the development of irreversibility. Furthermore, the release of active lysosomal proteases and other enzymes from damaged tissues into the blood stream, might also represent a potential factor in the lethal outcome of traumatic shock.

With respect to bacterial endotoxemia, there is even stronger reason to suppose that lysosomes play an important role. Even sublethal doses of endotoxin administered to young rabbits were found to liberate lysosomal hydrolases from the large granule fraction of liver within as little time as 5 minutes (4). Hence, the release of acid hydrolases into cell sap or surrounding fluids may constitute an important initiating factor in the sequence of pathological events observed in endotoxemia.

In order to investigate these possibilities, traumatic shock and endotoxin shock were produced in rats. Measurements were made of the release of beta glucuronidase and cathepsins from the large granule fraction of livers of shocked animals, and also of levels of circulating acid phosphatase and beta glucuronidase under these conditions. These enzymes have been shown to be associated with the lysosomes present in rat liver (7) and in other tissues. Several experiments were also carried out using animals rendered tolerant to traumatic injury by repeated conditioning, or else protected from the lethal effects of shock by pretreatment with cortisone, in order to observe the effects of tolerance and glucocorticoids upon the response of lysosomes during stress.

The results of these experiments suggest, first, that lysosomes play an important role in the deterioration of cell function during shock, and, second, that modifications of these particles induced either by prior conditioning of animals or by administration of glucocorticoids may be responsible for the increased resistance to shock exhibited in these situations.

Materials and Methods

1. Animals.—Female, Sprague-Dawley rats were obtained from Charles River Farms and were maintained on a diet of Purina laboratory chow and water, *ad libitum.* Animals weighing between 160 and 190 gm were used. Albino rabbits of the New Zealand strain weighing 1.5 to 2 kg were also employed in these studies.

2. Traumatic Shock in the Rat.—Rats were subjected to rotational trauma according to procedures described by Noble and Collip (8). The challenging dose of trauma was 650 revolutions, which represented an LD_{50} dose. One hour after drumming, or at death in the case of animals expiring before that time, blood was withdrawn from the abdominal aorta, under light ether anesthesia, for assay of plasma acid phosphatase and serum beta glucuronidase. Livers

were removed and placed on ice (but not frozen) prior to isolation of the large granule fraction of liver homogenates.

3. *Induction of Tolerance.*—Other groups of rats were rendered tolerant prior to receiving challenging doses of trauma. This was accomplished as follows: Animals were drummed 200 revolutions on days 1 and 2, 300 revolutions on days 3 and 4, 400 revolutions on days 5 and 6, 500 revolutions on the 7th day, were rested for 48 hours, and were then challenged on day 9. One hour post-challenge, blood samples and livers were removed as before.

4. *Endotoxin Shock.*—Endotoxin shock was produced in rats by intraperitoneal injection of 2.5 mg (an LD_{50} dose) of a purified preparation of *Salmonella enteritidis* endotoxin (lot 116142, Difco Co., Detroit). Blood samples and livers were obtained as usual, although in some experiments acid phosphatase of plasma was determined on blood collected 4 hours after injection of toxin.

5. *Bowel Ischemia Shock.*—Rabbits were anesthetized with intravenous sodium pentobarbital (nembutal, Abbott Laboratories, Chicago) (30 mg/kg) and were subjected to 90 minutes of superior mesenteric artery ligation by a technique described before (9). Samples of hepatic portal venous blood or femoral venous blood were obtained at intervals before, during, and after the period of arterial occlusion, for assay of acid phosphatase.

6. *Plasma Acid Phosphatase Assay.*—Acid phosphatase of plasma was assayed according to the method of Bessey, Lowry, and Brock (10). Aliquots (0.1 ml) were incubated with disodium *p*-nitrophenyl phosphate substrate in 0.00375 M concentration. The incubations were carried out at 37°C in 0.1 M citrate buffer at pH 4.8 containing 0.001 M Mg^{++}. Acid phosphatase activity was expressed as millimoles of *p*-nitrophenol released per hour at 37°C per 1000 cc of plasma.

7. *Acid Phosphatase Correction Factor (Hemoglobin Assays).*—Since erythrocytes also contain an acid phosphomonoesterase which is highly active against phenyl-phosphates as substrate (11), and since considerable intravascular blood cell breakage can occur during traumatic shock, it was felt that some component of the increment in plasma acid phosphatase in shocked animals might be due to the accompanying destruction of erythrocytes. Although enzyme released from blood cells during shock also constitutes an important derangement, an attempt was nevertheless made to distinguish between this source of acid phosphatase and enzyme released from damaged tissues. A method was devised for the estimation of plasma acid phosphatase derived from blood cells in shocked animals. Blood obtained from normal animals was pooled and divided into several fractions. Each fraction was exposed to an arbitrary degree of mechanical trauma by aspiration and ejection through a fine bore hypodermic needle. Different degrees of hemolysis occurred in the various samples as a result of this treatment. Acid phosphatase assays were then performed on the plasma supernates of these samples. The values obtained were corrected for the original enzyme content of unhemolyzed plasma by subtracting the reading obtained from an untreated sample of blood. Thus, enzyme released as a result of hemolysis *per se* was determined. The same series of samples was simultaneously assayed for hemoglobin content by the method of Shinowara (12). On the basis of this procedure, repeated several times, a calibration curve was established relating hemoglobin content and red cell acid phosphatase content of plasma samples. Thereafter, hemoglobin determinations were carried out simultaneously with enzyme assays on all samples of shock plasma. In the data to be presented, all the plasma acid phosphatase values have been corrected for the maximal contribution from broken blood cells (calculated from the hemoglobin-enzyme calibration curve). Plasma acid phosphatase activity which could be attributed to enzyme release from damaged blood cells was generally 20 per cent or less of the total activity of the sample.

8. *Serum Beta Glucuronidase Assay.*—Serum beta glucuronidase was determined by the method of Fishman *et al.* (13) on 0.2 ml of fresh serum. Activity is expressed as micrograms of phenolphthalein released per 100 ml of serum per hour at 37°C.

9. Preparation of a Large Granule Fraction from Rat Liver and Measurement of the Release of Beta Glucuronidase and Cathepsin.—Methods for preparing a large granule fraction of rat liver were identical with those described in the accompanying paper (4). Livers from normal animals, shocked animals, and animals rendered tolerant prior to shock were used. Briefly, the test procedures involved the exposure of a large granule fraction of rat liver to mercury arc irradiation for 40 minutes at 37°C. Other aliquots of the fractions were incubated at 37°C. for 40 minutes without irradiation. Incubation, by itself, results in the gradual release of lysosomal enzymes from the particles; while ultraviolet irradiation markedly accelerates this phenomenon (14). The free activity of cathepsins and of beta glucuronidase in the supernates of the incubated fractions was measured as an index of the rate of release of these enzymes from lysosomes under the different conditions described. Liver lysosome fractions from shocked animals were compared with those obtained from normal animals and, in this way, it was possible to determine the effects of trauma or of endotoxin upon the "stability" of these particles. In addition, total and free activities of cathepsins and beta glucuronidase were measured prior to incubation or irradiation of the fractions. This made it possible to estimate the degree of release of enzyme from hepatic lysosomes occurring as a direct result of trauma or administration of endotoxin. The data therefore include lysosomal "breakage" after shock, as well as changes in lysosomal "fragility." Cathepsins were determined by the method of Anson (15), while beta glucuronidase was measured according to the method of Fishman (13).

RESULTS

I. Appearance of Lysosomal Enzymes in the Blood Stream during Shock

(*a*) *Traumatic Shock in Rats.*—The data in Fig. 1 show that, 1 hour after an LD_{50} dose of rotational trauma, levels of plasma acid phosphatase were two and one-half times greater than normal. At the same time, average beta glucuronidase levels were also increased to two and one-half times the normal serum titer of this enzyme (see Table I).

(*b*) *Bowel Ischemia Shock in Rabbits.*—Similar increases in plasma acid phosphatase occurred in rabbits during shock induced by temporary occlusion of the superior mesenteric artery. These alterations in enzyme levels were detected both in femoral and hepatic portal venous blood (see Fig. 2).

(*c*) *Endotoxin Shock in Rats.*—As with trauma, intraperitoneal injection of an LD_{50} dose of bacterial endotoxin (*S. enteritidis*) caused marked elevation in levels of circulating acid phosphatase and beta glucuronidase. Four hours after the injection, acid phosphatase was increased twofold (Fig. 3). In the case of beta glucuronidase, measurements were made 1 hour after the administration of the toxin, and Table I shows that levels of this enzyme were also increased approximately twofold, at this time.

II. Alterations of Hepatic Lysosomes during Shock

Attempts were made to correlate the observed increases in blood levels of acid phosphatase and beta glucuronidase in shocked animals with release of these enzymes from hepatic lysosomes after shock. It was found, however, that liver acid phosphatase measured by the Bessey method was particularly

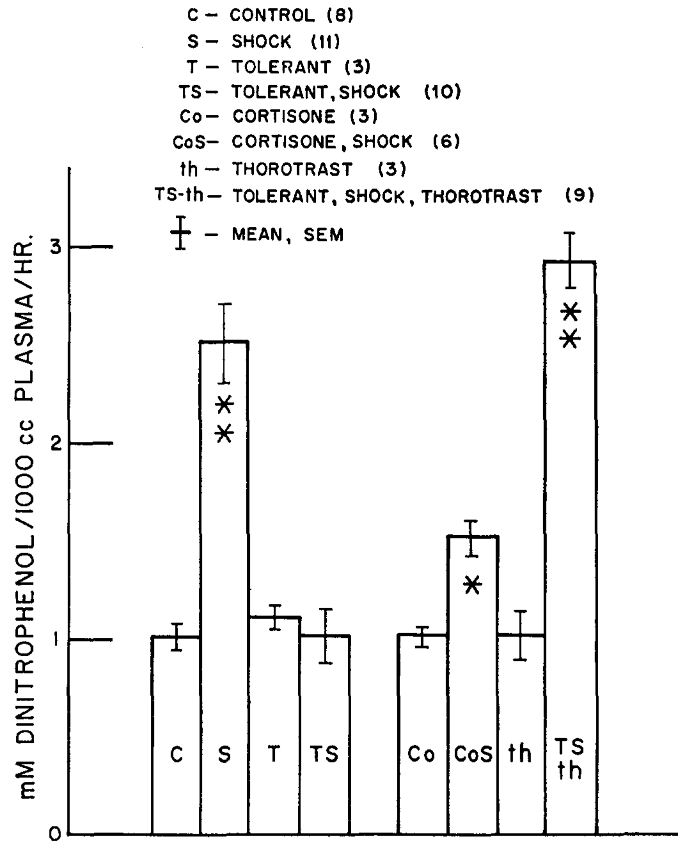

FIG. 1. Changes in plasma acid phosphatase in rats during traumatic shock. All enzyme values have been corrected for erythrocyte–acid phosphatase released as a result of intravascular hemolysis. One enzyme unit equals 1 millimole of dinitrophenol released per hour, per 1000 cc plasma, at 37°C. Experimental groups are identified within the figure.

TABLE I

Levels of Serum Beta Glucuronidase in Shock

Experiment	No. of rats	Enzyme units*
Control	5	310 ± 34
Endotoxin	4	593 ± 62
Traumatic shock	4	785 ± 83
Tolerant plus traumatic shock	4	725 ± 44

* 1 unit = 1 microgram of phenolphthalein released per 100 ml. serum per hour.

labile to ultraviolet irradiation and mechanical disintegration. This enzyme therefore could not be used in studies on hepatic lysosomes during shock. Instead, the data reported below are based on measurements of beta glucuronidase, and of another group of lysosomal enzymes, the cathepsins.

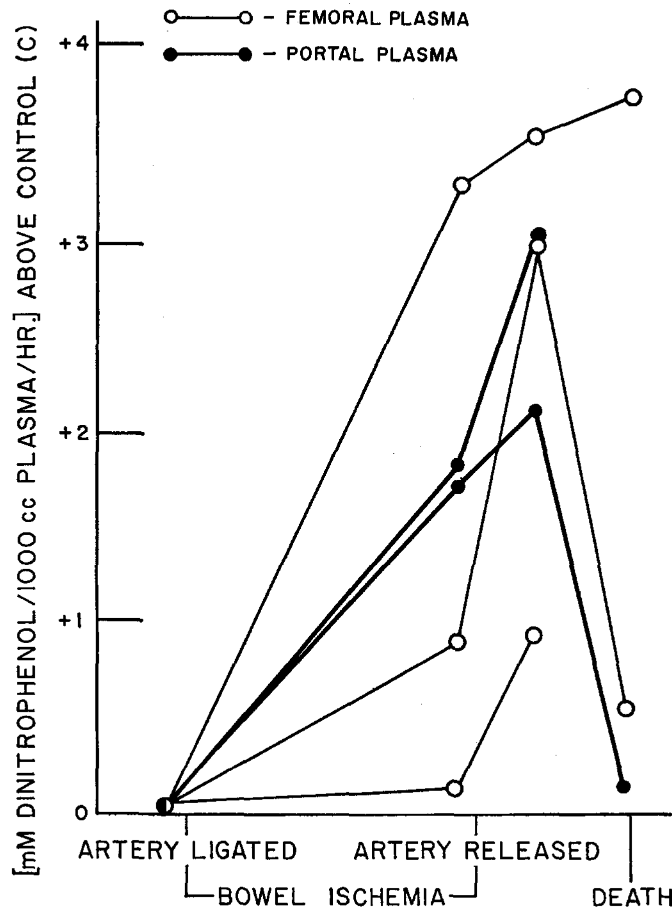

FIG. 2. Changes in plasma acid phosphatase in rabbits during traumatic (bowel ischemia) shock. Increases in enzyme units above starting (control) values are shown for each animal. Control levels were measured just prior to ligation of the superior mesenteric artery. Arterial ligation was maintained for 90 minutes in each case.

(*a*) *Traumatic Shock in Rats.*—As expected, a decrease in total activity of beta glucuronidase was detected in homogenates of livers obtained from shocked animals. These results are presented in Fig. 4. In the same figure, total cathepsin activities of homogenates of normal and traumatized rats are also shown, and it can be seen that only a slight decrease in this enzyme was observed after shock.

However, the significance of the decrease in total liver beta glucuronidase produced by trauma was reinforced by experiments in which the free activity of this enzyme in livers of shocked rats was found to be increased (see Fig. 5).

Studies on the *in vitro* release of acid hydrolases from the large granule fraction of liver also support the view that lysosomes undergo significant alterations

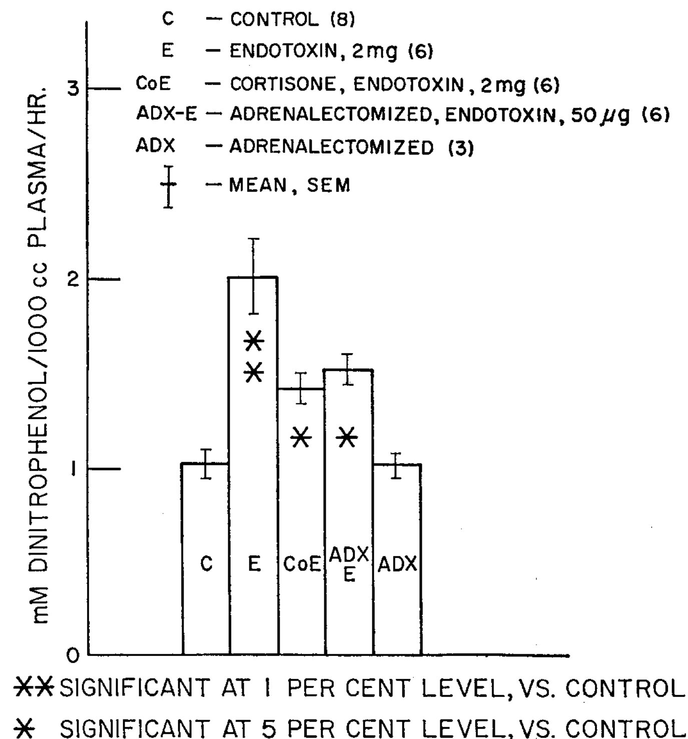

FIG. 3. Changes in plasma acid phosphatase in rats during endotoxemia. Experimental groups are identified within the figure.

during traumatic shock. For example, Fig. 6 shows that a greater release of cathepsins occurred when suspensions prepared from shocked rats were incubated at 37°C, than was the case with suspensions obtained from unshocked controls. However, the release of cathepsins induced by mercury arc irradiation was only slightly greater in the case of particle suspensions obtained from shocked animals (Fig. 6). These differences between particles of shocked and unshocked rats were more pronounced when release of beta glucuronidase activity was studied under identical circumstances (Fig. 7).

(*b*) *Endotoxin Shock in Rats.*—In this form of shock, similar correlations were found between increased levels of circulating lysosomal enzymes and

changes in total and free activity of lysosomal enzymes in liver homogenates. Considerable reductions in the total activity of liver cathepsins and beta glucuronidase occurred 1 hour after the administration of bacterial endotoxin (see Fig. 4). Furthermore, as shown in Fig. 5, a significantly increased quantity of free beta glucuronidase activity was present in these same livers.

Release of acid hydrolases from large granule suspensions obtained from rats given endotoxin was also markedly affected. Fig. 6 shows that incubation of these suspensions at 37°C resulted in a slightly greater release of cathepsins in the case of endotoxin-treated animals than in controls. This difference be-

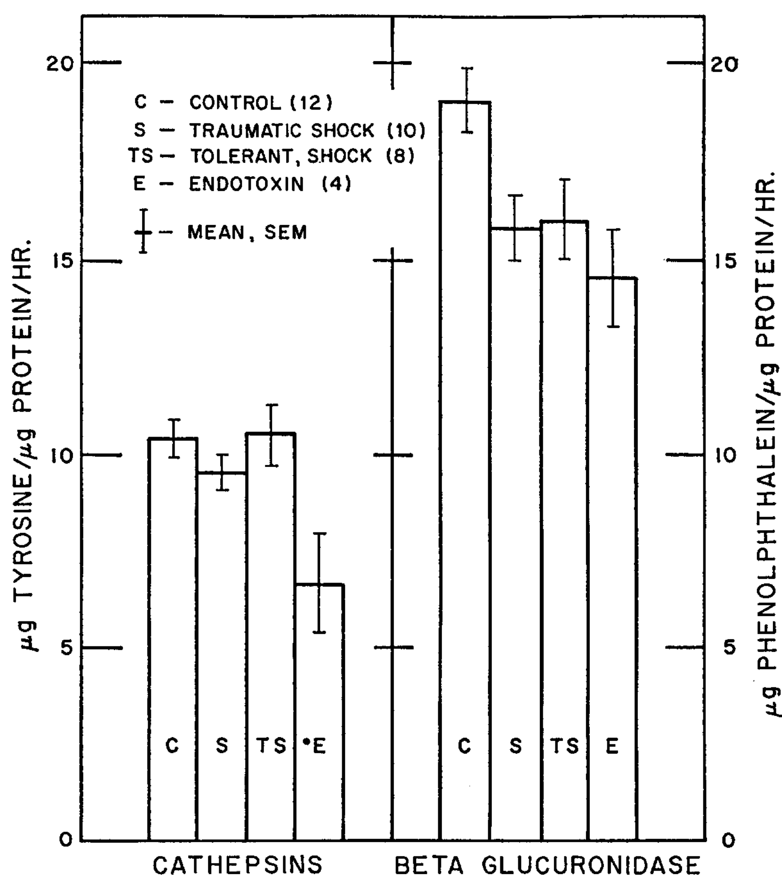

FIG. 4. Total activity of cathepsins and beta glucuronidase in rat liver homogenates. Unshocked animals are compared with conventional and tolerant animals exposed to trauma and with endotoxin-treated animals. Units of enzyme activity are expressed as micrograms of tyrosine and phenolphthalein, for cathepsins and beta glucuronidase respectively, released per microgram of protein per hour.

tween the large granule suspensions of normal and endotoxin-treated animals was even more apparent when studies were made of release of cathepsins from particles exposed to mercury arc irradiation. These data are also shown in Fig. 6, and it is clear that suspensions obtained from endotoxin-treated rats showed greater than normal fragility during irradiation. Similar results were obtained in studies on the release of beta glucuronidase from incubated and irradiated suspensions (Fig. 7).

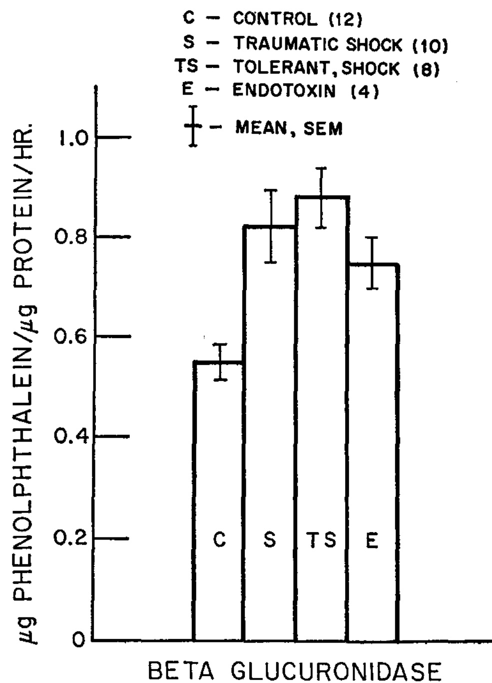

FIG. 5. Free activity of beta glucuronidase in rat liver homogenates. Unshocked animals are compared with conventional and tolerant animals exposed to trauma and with endotoxin-treated animals.

III. Effect of Tolerance and Cortisone upon Blood Levels of Lysosomal Enzymes during Shock

(*a*) *Traumatic Shock in Rats.*—Earlier observations (16) have shown that exposure of animals to repeated small doses of trauma will result in a tolerant state characterized by resistance to ordinarily lethal challenge. When animals were rendered tolerant and then challenged with an LD_{50} dose of trauma, blood levels of acid phosphatase were not elevated 1 hour later, although normal rats showed a twofold or greater increase after shock. These results are presented in Fig. 1 (compare TS group with C and S groups).

Intraperitoneal injection of 10 mg of cortisone (cortisone acetate, Philadelphia Ampoule Laboratories, Philadelphia) into rats 30 minutes before challenge with an LD_{50} dose of trauma, significantly decreased the release of acid phosphatase into the circulation. These results are also shown in Fig. 1 (CoS

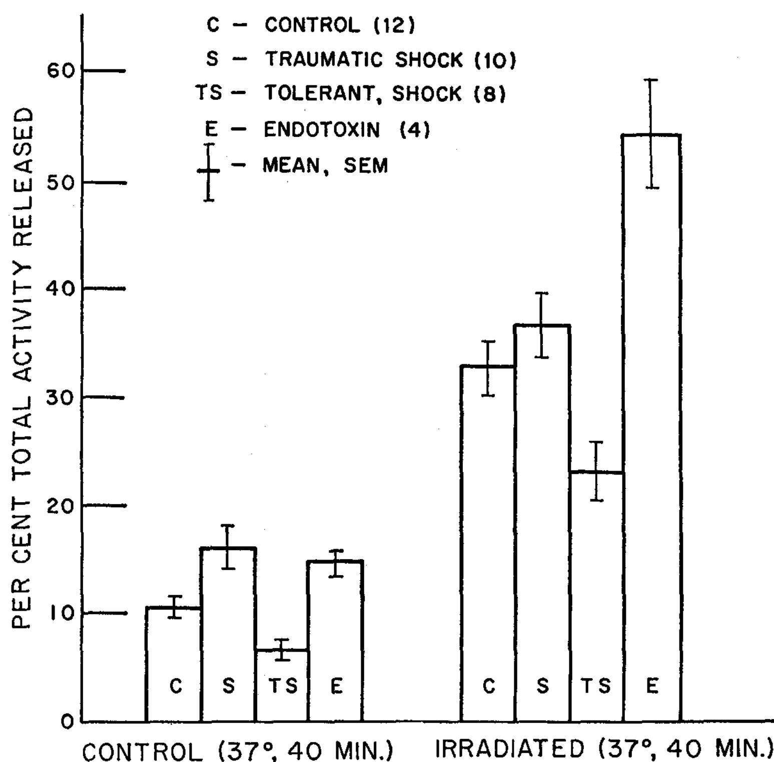

FIG. 6. Release of free catheptic activity from the large granule fraction of rat liver homogenates during simple incubation (control) and ultraviolet-irradiated incubation of these fractions. Catheptic activity released is expressed as per cent of total enzyme activity in the fraction. Large granule fractions prepared from unshocked rats (C) are compared with fractions prepared from conventional and tolerant animals after trauma, and with fractions from endotoxin-treated animals.

group). Thus, two protective regimens, pretreatment with a glucocorticoid and induction of natural tolerance by preconditioning, had a similar inhibitory effect on the release of lysosomal enzymes into the circulation during traumatic shock.

The trauma-resistant state can be abrogated by a single, large dose of colloid injected intravenously (17). This circumstance provided an opportunity to reaffirm the reliability of the lysosomal enzyme data by noting whether

colloid-treated, tolerant animals would respond to trauma by releasing acid phosphatase into the circulation in the same manner as did normal animals. Rats were rendered tolerant by the procedures described previously. Half of these animals were given intravenous injections of 100 mg of thorotrast (colloidal thorium dioxide, Testagar & Co., Detroit) 2 hours before receiving an

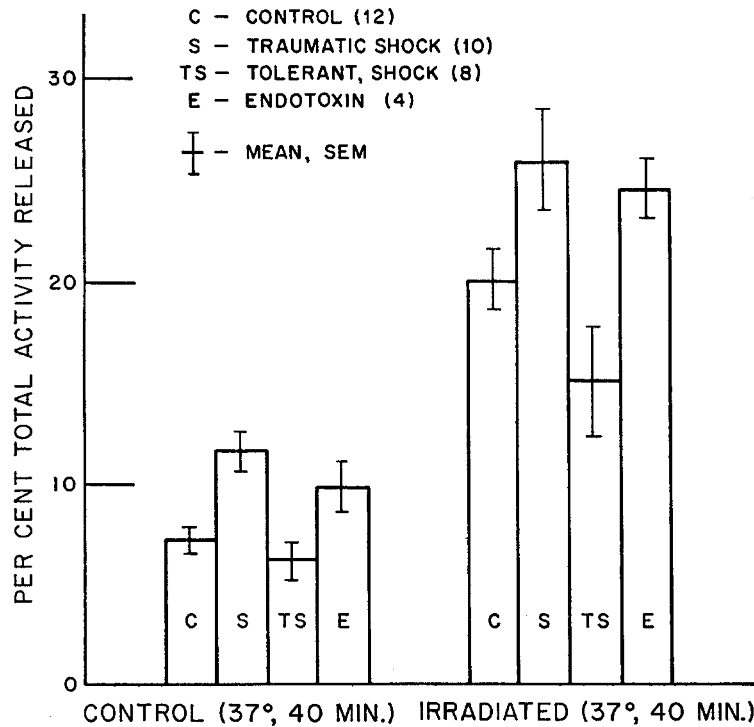

FIG. 7. Release of free beta glucuronidase activity from control-incubated and ultraviolet-irradiated large granule fractions of rat liver homogenates. Control, shocked, and tolerant groups are compared as before.

LD_{50} dose of trauma. The others received an equivalent volume of saline. One hour after trauma, plasma acid phosphatase levels were elevated to three times control values in the tolerant animals which received colloid prior to shock (TS-th group), while the tolerant group treated with saline showed no increase in plasma enzyme (TS group). These data are presented in Fig. 1. Also summarized in the figure are plasma enzyme levels observed in a selected number of tolerant rats treated with thorotrast alone, but not subjected to shock (th group). These results indicate that thorotrast, by itself, was not responsible for the change in plasma acid phosphatase observed in the shocked group.

In the experiments on tolerance, serum beta glucuronidase was also measured. In contrast to the findings with acid phosphatase, high levels of this enzyme were encountered in tolerant animals after trauma (see Table I). The difference in behavior of acid phosphatase and beta glucuronidase during shock in tolerant animals was resolved by the discovery that circulating acid phosphatase activity decays at a rapid rate; whereas beta glucuronidase activity persists in the blood stream for much longer intervals. Thus when twelve rats were challenged with an LD_{50} dose of trauma, and four animals were sacrificed an hour later, the mean values of the two enzymes were 2.5 units for acid phosphatase and 785 units for beta glucuronidase. Forty-eight hours later, in four sacrificed animals, acid phosphatase had returned to control levels (1.0 unit); whereas beta glucuronidase (516 units) was still above control values (310 units). These observations make it reasonable to assume: first, that increases in beta glucuronidase (occurring in the early stages of conditioning as a result of the initial episodes of trauma) would persist and lead to an over-all accumulation of this enzyme in the circulation of tolerant rats. Second, this cumulative increase of enzyme could mask the effect of tolerance upon newly released beta glucuronidase following final challenge. Acid phosphatase, on the other hand, would have returned to control levels in tolerant animals prior to their challenge. Assay of this factor, therefore, would permit a sensitive measure of newly released lysosomal enzyme at the time of final challenge. For these reasons, only acid phosphatase has been considered in the discussion of the release of lysosomal enzymes into the circulation of tolerant animals.

(*b*) *Endotoxin Shock in Rats.*—As was the case with traumatic shock, rats pretreated with 10 mg of cortisone (intraperitoneally) 30 minutes before challenge with an LD_{50} dose of bacterial endotoxin, exhibited smaller increases in plasma acid phosphatase than were encountered after endotoxin treatment alone (see Fig. 3).

In view of this effect of exogenous cortisone, an experiment was designed to investigate the effect of endogenous corticosteroids on the rate of release of acid phosphatase following endotoxin. Since minute fractions of the conventional lethal dose of endotoxin are capable of causing death in adrenalectomized mice (18), measurements were made of the release of acid phosphatase in adrenalectomized rats given one fiftieth of an LD_{50} dose of endotoxin. Rats were bilaterally adrenalectomized (under light ether anesthesia) and maintained for 7 days thereafter on 0.85 per cent sodium chloride in the drinking water. The administration of 50 μg of endotoxin to these animals on the 8th postoperative day caused a significant elevation in plasma acid phosphatase, although enzyme levels were not as high as in intact rats given 2.5 mg of the toxin preparation. Enzyme levels were in the control range in adrenalectomized rats not receiving endotoxin. The data are included in Fig. 3.

IV. Effect of Tolerance upon Alterations of Hepatic Lysosomes in Traumatic Shock

As in the case of conventional animals (see Results, section II), the total beta glucuronidase activity in homogenates of livers from tolerant rats decreased following trauma (Fig. 4). Moreover, a similar increase in free activity

of beta glucuronidase in these same liver homogenates (shocked animals) occurred despite prior conditioning (Fig. 5).

However, the increased fragility of hepatic lysosomes (as tested *in vitro*) in normal rats following trauma was not observed in the case of tolerant animals. Particles harvested from tolerant animals showed an increased resistance to *in vitro* lysis. Thus, in the incubated large granule fractions of livers obtained from shocked, tolerant animals, the release of free catheptic and beta glucuronidase activity was much slower than it was in incubated suspensions obtained from shocked, conventional rats—and was even slower than in suspensions prepared from control rats not subjected to shock! This was true both for spontaneous release of enzymes (incubation at 37°C for 40 minutes),as well as for the additional release effected by ultraviolet irradiation (see Figs. 6 and 7).

DISCUSSION

The results reported here show that shock induced by two agencies, trauma and bacterial endotoxin, was consistently associated with an elevation in plasma acid phosphatase and serum beta glucuronidase. It is especially significant that these enzymes were released into the circulation in a predictable manner when the status of the test animal was modified so as to either increase or decrease susceptibility to lethal shock. Thus, enzyme release did not occur in trauma-tolerant animals and was significantly depressed following cortisone pretreatment. On the other hand, when tolerance was abrogated by colloids, challenge then resulted in the liberation of excessive amounts of enzymes into the circulation, as developed as routine in conventional rats subjected to shock. Small doses of endotoxin produced a disproportionately enhanced enzyme response in adrenalectomized rats.

The findings suggest that the appearance of lysosomal enzymes in the circulation during shock may be intimately associated with the lethal progression of the syndrome. Certainly, changes in these enzymes during shock should not be regarded as an epiphenomenon; nor should they be classed along with enzymes of the transaminase or dehydrogenase type, the behavior of which cannot serve as a prognostic index of the lethality of the shock procedure. This general thesis is further strengthened by the data indicating changes in total and free activity of cathepsins and beta glucuronidase in liver homogenates prepared from shocked animals, and an increased fragility of the large granule fraction of livers of these animals.

It is plausible to assume that the liberation of hepatic lysosomal enzymes by trauma and by endotoxin, as well as the apparent release of these enzymes from cells of the small intestine of rabbits during bowel ischemia, would serve as contributory factors to the irreversible changes at the cellular level characteristic of shock. It should be emphasized that, while our data showing release of beta glucuronidase and acid phosphatase from hepatic lysosomes into the

circulation support the concept of lysosomal disruption during shock, the authors do not suggest that irreversibility results from the hydrolytic activities of these enzymes alone. Rather, one should consider de Duve's general thesis (1) that all lysosomal hydrolases are, more or less, released together during non-selective disruption of lysosomal particles. Thus, if it is assumed that intracellular liberation of free proteases (cathepsins) and nucleases (DNAase, RNAase) also occurs during shock, then the hypothesis linking lysosomal "rupture" to the development of irreversibility becomes especially attractive. Since evidence has already been presented in this report for the increased release of cathepsins in livers of shocked animals, the assumption discussed above is not an unlikely one. In the case of endotoxin shock, there are sound grounds for suggesting that the intracellular activation of lysosomal enzymes plays an early role in the process. This point has been discussed elsewhere (4).

The finding of increased lysosomal stability in liver preparations from preconditioned rats provides an attractive hypothesis to explain the protection afforded by tolerance. If disruption of lysosomes, *in vivo*, represents an important determinant of the response of animals to stress (as the data seem to indicate), then the heightened resistance of these particles in conditioned animals might help to account for the phenomenon of tolerance.

Cortisone treatment, like the induction of tolerance, has been shown to significantly increase the resistance of hepatic lysosomes to *in vitro* lysis (4). The data presented in this report show that this drug also reduces the release of acid phosphatase into the circulation in traumatized and endotoxin-treated rats. It is therefore possible that some of the pharmacological properties of cortisone may result from the action of this steroid upon lysosomes.

The results of these experiments by themselves do not unequivocally relate the behavior of lysosomes to the pathogenesis of experimental shock in normal and tolerant animals. However, the findings do provide a working hypothesis for future investigation, and at least an initial approach to a basic aspect of the problem.

SUMMARY

Fatal shock was produced in animals by drum trauma, temporary occlusion of the superior mesenteric artery, and bacterial endotoxin. Measurements were made of release of beta glucuronidase and cathepsins from the large granule fractions of livers, and of levels of circulating beta glucuronidase and acid phosphatase in these animals. Experiments were also carried out with animals rendered tolerant by previous exposure to sublethal amounts of trauma or by pretreatment with cortisone.

The results show that release of beta glucuronidase and cathepsins from the large granule fraction of liver was increased during traumatic and endotoxin shock in the rat. Similarly, circulating levels of acid phosphatase and beta

glucuronidase were increased during traumatic shock in rats and rabbits, and during endotoxin shock in rats. The data also indicate that tolerance to traumatic injury, induced by prior conditioning, prevented the increase in levels of circulating acid phosphatase normally observed after stress, and may have been associated with an increased stability of hepatic lysosomal particles. In addition, cortisone, which appears to "stabilize" hepatic lysosomes *in vivo*, also reduced the increase in plasma acid phosphatase brought about by endotoxin and trauma.

From the foregoing observations, it is suggested that: (*a*) Disruption of lysosomes and release of their contained enzymes in free, active form may occur in liver and intestine of shocked animals. (*b*) The activation of lysosomal hydrolases within cells and their release into the circulation may play an important role in exacerbating tissue injury and accelerating the development of irreversibility during shock. (*c*) The increased stability of lysosomes of tolerant and of cortisone-treated animals may constitute an important component of the resistance of these animals to shock.

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