Passive Transfer Protection in Traumatic Shock* (32706)

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It is well known that reticuloendothelial system (RES) depression through carbon "blockade" temporarily increases the susceptibility to subsequent hemorrhagic or traumatic shock (1–3) whereas RES stimulation, notably with zymosan injection, increases resistance to the same stresses (2,4). These findings suggest that the physiological state of the RES as measured by phagocytic capacity constitutes an important determinant of shock resistance. Glucan injection, on the other hand, also produces marked hyperphagocytosis but does not afford shock protection (4); it appears, therefore, that RES stimulation is not inevitably effective against shock. It seemed possible that another RES mechanism, perhaps immunological, might be operative in the shock-protective effect of zymosan.

Previous studies indicated that the RES plays an important role in antibody formation (5) and as a defense against infection (6). Thus, shock resistance induced by zymosan may involve the production of antibodies or other immune factors and the difference between zymosan and glucan in this regard may be on an immunological basis. Zymosan increases resistance to *E. coli* infections (7), enhances the bactericidal power of serum against gram-negative organisms (8), produces heat-stable antibodies (9), alters properdin levels *in vivo* (10) and interacts with properdin *in vitro* (11). Furthermore, zymosan and glucan both enhance the primary and secondary immune responses to particulate antigens (12,13).

If circulating endotoxin exerts a major deleterious effect during shock (14,15), an immune mechanism for endotoxin inactivation could also conceivably be a factor in shock protection. Freedman has reported that tolerance to bacterial endotoxin can be achieved by passive transfer of serum from endotoxin-tolerant donors to normal recipient animals (16). This approach has been used in the present study to evaluate the role of the immune response to zymosan and glucan injection in subsequent traumatic shock.

Materials and Methods. Drum shock. Male Sprague-Dawley albino rats weighing 250–300 gm were maintained on a standard diet with water *ad libitum* and were given a single iv injection of either 100 mg/kg zymosan (Standard Brands, Stamford, Conn., Lot No. 9B551) or glucan (Standard Brands, Stamford, Conn., Lot No. 1F5500) or an equivalent volume of pyrogen-free saline. Seventy-two hours later, the animals were tumbled in a modified Noble-Collip drum at 33 rpm for a total of 425 rev. using a technique previously described (4). The rats were closely observed and those living after 24 hours were considered survivors. Gross postmortem examinations were performed on all fatalities; animals dying with the characteristic intestinal congestion and luminal hemorrhage but with no other apparent visceral organ damage were classified as shock mortalities; the data were analyzed with the chi-square test.

Serum Preparation. Pooled serum was prepared from the blood of zymosan, glucan, saline-injected and uninjected rats. Unanesthetized rats were bled via cardiac puncture, and the serum was divided into three parts, one for carbon clearance tests, one for antibody and complement determination, and the third for passive transfer to other rats. In another series, anesthetized rats (Nembutal, 12–15 mg, ip) were bled via the abdominal aorta and the serum was utilized for antibody studies.

Passive transfer drum shock. Normal Sprague-Dawley rats received one iv injection of 0.4 ml of whole serum/100 gm of body wt. obtained from unanesthetized rats. Thirty min following passive transfer, the recipient rats were either drum shocked at 33 rpm (425 or 475 total rev.) or evaluated for carbon clearance capacity.

Carbon clearance. The phagocytic rate was

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TABLE I. Effect of Zymosan and Glucan Injection on Shock Resistance and RES Organ Weights in the Rat.

Group	N	% Shock mortality	% Body wt. (Mean ± SD)		
			Liver	Spleen	Lung
Saline	35	74	4.33 ± .37	0.36 ± .11	0.62 ± .19
Zymosan	18	22[a]	4.84 ± .51[b]	0.65 ± .13[b]	0.73 ± .16[a]
Glucan	16	88	5.28 ± .45[b]	0.66 ± .11[b]	1.32 ± .20[b]
			Shock was induced 72 hours postinjection		

[a] p value <0.05 when compared to saline control.
[b] p value <0.001 when compared to saline control.

measured with a modification of the technique of Biozzi *et al.* (17). Collodial carbon (C11/1431a Pelikan, Gunther-Wagner, Hanover, Germany) in gelatin, 8 mg/100 gm of body wt., was injected iv into unanesthetized rats and a timed series of 0.025-ml blood samples was taken from the tail vein, hemolyzed in 2.5 ml of 0.01% sodium carbonate, and the carbon concentration was determined spectrophotometrically at 675 mμ. The phagocytic capacity was expressed as the phagocytic index, K (17). Wet weights of the liver, spleen, and lung were determined and the data analyzed using Student's t test.

Antibody determination. Pooled serum from zymosan, glucan, and saline-treated rats was absorbed twice for 10 min each at 0°C with 3 ml of packed sheep erythrocytes for every 100 ml of serum. The absorbed serum was heated at 56°C for 30 min and analyzed with a qualitative complement fixation test modified from Kabat and Mayer (18).

1) *Hemolysin stock solution.* Two ml of glycerinized antisheep hemolysin (Sylvania Chemical Co., Orange, N.J.) were added to a solution containing 4 ml of 5% phenol and 94 ml of Kolmer saline. The 1:100 stock solution was kept at 4°C and discarded when a precipitate or a drop in hemolysin titer occurred; the hemolysin was titrated according to the method of Kabat and Mayer (18).

2) *Sensitized sheep erythrocytes.* Sheep erythrocytes were stored overnight in isotonic saline containing 0.01 M EDTA and washed at least three times with 0.01 M EDTA in gelatin veronal buffer (GVB). The sheep cell suspension was adjusted to 1.0×10^9 cell/ml and mixed with an equal volume of stock hemolysin diluted to 1:1600 with 0.01 M EDTA/GVB. The reaction mixture was incubated at 37°C and then at 0°C for 30 min each and washed once with 0.01 M EDTA/GVB and twice with GVB.

3) *Complement (C').* Pooled serum from guinea pigs was absorbed twice for 10 min at 0°C with 3 ml of packed sheep erythrocytes/100 ml of serum, three times for 15 min at 17°C with 2 mg of zymosan and once for 15 min at 17°C with 2 mg of glucan. Preliminary experiments indicated that multiple absorptions resulted in 85–95% removal of anticomplementary effects due to zymosan and glucan as determined by quantitative residual complement titers (19); the serum was analyzed for complement (18) and used for antibody determinations.

Complement titration. Unheated and absorbed serum from zymosan, glucan, and saline-treated rats was also analyzed for complement (18). The sensitized sheep cells were prepared as outlined for the antibody determinations and the resultant titers were expressed in terms of complement hemolytic fifty units ($C'H_{50}$ units).

Results. Effect of zymosan and glucan on traumatic shock mortality and carbon clearance. Both zymosan and glucan produced RES stimulation as evidenced by significant increases in wet weights of the primary RES organs (Table I) but shock mortality was significantly lower only in the zymosan-treated rats. These findings were similar to those reported by Filkins, *et al* (4).

Effect of passively transferred serum on traumatic shock. Whole serum from both

TABLE II. Effect of Passively Transferred Serum from Zymosan, Glucan, and Saline-Injected Rats on Traumatic Shock Mortality in Recipient Rats.

Group	No.	Shock mortality
Control serum	29	17/29 (59)[b]
Zymosan serum	28	8/28 (29)[a]
Control serum	30	18/30 (61)
Glucan serum	29	9/29 (31)[a]

[a] p value <0.05 when compared to respective control serum group.

[b] Percentages are given in parentheses.

zymosan- and glucan-treated rats produced a significant reduction in shock mortality of normal recipient rats 30 min following passive transfer (Table II). Heat-stable antibody, however, was present only in serum from zymosan-treated rats and then only when zymosan was employed as the antigen in the antibody determinations; on the other hand, only the serum from glucan-treated rats had elevated complement titers compared to the control groups (Table III).

Relationship of antibody levels and traumatic shock mortality. A total of four series of experiments involving passive transfer of serum from saline and zymosan-injected rats were done in the course of the investigation in order to determine the consistency of the passive transfer effect as well as the relationship between serum antibody content and shock mortality. Passive transfer of serum from zymosan-treated animals to normal recipient rats produced a definite shock protection in Series 1 but a lesser tendency toward protection in Series 2, 3, and 4 (Table IV). If all series were combined, the zymosan effect was statistically significant at a confidence level between 98 and 99%.

The lesser protective effects against drum shock observed in Series 3 and 4 (Table IV) were associated with minimal antibody titers in the zymosan-treated rats, particularly in anesthetized animals. The tendency towards lower titers in the unanesthetized animals (Table IV) was an unexpected but rather consistent finding. It may be related to a differential effect of anesthesia on plasma volume and/or to a splenic release of more concentrated antibody in the anesthetized animal.

TABLE IV. Relationship of Serum Antibody Titers and Passive-Transfer Drum Shock Mortality in Saline and Zymosan-Injected Rats.

Series	Shock mortality	Antibody titers: Anesthetized	Antibody titers: Unanesthetized
1. Control serum	17/29 (59)[b]	0	0
Zymosan serum	8/28 (29)[a]	200	50
2. Control serum	14/21 (67)	0	0
Zymosan serum	9/23 (39)	160	80
3. Control serum	12/20 (60)	0	0
Zymosan serum	9/20 (45)	80	50
4. Control serum	16/20 (80)	0	0
Zymosan serum	15/20 (75)	10	10

Stress in Series 1 was 425 total rev., in Series 2, 3, and 4 475 rev.

[a] p value <0.05 when compared to control serum group.

[b] Percentages are given in parentheses.

TABLE III. Effect of Zymosan and Glucan on Serum Antibody and Complement Titers.

Group	No. of expts.	Antigen	Antibody titer	Complement titer ($C'H_{50}$ units/ml)
Zymosan	5	Zymosan	120–200	22.2
		Glucan	0	
Glucan	3	Zymosan	0	60.2
		Glucan	0	
Saline	2	Zymosan	0	20.4
		Glucan	0	
Normal serum (uninjected)	5	Zymosan	0	24.8
		Glucan	0	

TABLE V. Effect of Passively Transferred Serum on RES Stimulation.

Group	No. of rats	Antigen	Antibody titer	Phagocytic index, K (Mean ± SEM)
Uninjected control	8	Zymosan	0	0.0200 ± .0013
		Glucan	0	
Saline-injected control serum	6	Zymosan	0	0.0246 ± .0050
		Glucan	0	
Zymosan serum	8	Zymosan	60	0.0301 ± .0034[a]
		Glucan	0	
Glucan serum	8	Zymosan	10	0.0249 ± .0028
		Glucan	0	

[a] p value <0.400 when compared to saline serum animals; p value <0.025 when compared to uninjected control animals.

The reason for the variation in antibody titers in the different series is not clear but the immune responsivity of the animals may account for the differences. Animals demonstrating high antibody levels may have a high titer of natural antibody against zymosan (20) and the zymosan injection may have evoked a secondary immune response. The low antibody levels in Series 3 and 4 could conceivably reflect a primary immune response in the rats with minimal antibody levels prior to zymosan injection. Since natural antibody titers against zymosan were not determined, we have no direct evidence on this point.

Effect of passively transferred serum on RES stimulation. Freedman has reported that passive transfer of endotoxin tolerance in rabbits was associated with RES stimulation of the recipients (21); he suggested therefore that the functional state of the RES was causally related to the phenomenon of endotoxin tolerance. In our animals, passive serum transfer from either zymosan- or glucan-injected rats had no effect on carbon clearance 30 min after the transfer compared to animals receiving transfer of saline-injected control serum (Table V). In the zymosan group there was, however, an elevation of the K values when compared to uninjected controls suggesting that nonspecific and immune factors in serum from zymosan-treated rats enhanced carbon clearance to some extent, perhaps through an opsonic effect. The serum from unanesthetized zymosan-treated animals also had high antibody titers. It appears, therefore, that the level of phagocytic activity does not correlate well with the shock-protective effect of passively transferred serum. The difference between these results and those of Freedman may be related to the different properties of endotoxin and zymosan or perhaps to the time element between passive transfer and carbon clearance determinations which in Freedman's study was 3 hours.

Discussion. RES stimulation with zymosan, a cell wall extract of the yeast *Saccharomyces cerevisiae,* was again associated with a protective effect in traumatic shock as has been previously reported (2,4). Glucan, a polysaccharide component of zymosan, likewise produced a marked RES stimulation but did not afford protection to the same stress. However, passive transfer of serum from both zymosan- and glucan-treated rats increased the resistance to normal recipient rats to traumatic shock. The inability of glucan-treated rats to withstand drum shock, even though passive serum transfer from these animals protected normal recipients against the same stress, may be related to the pathological effects of glucan injection. Glucan induces marked hyperplasia of the RES elements of the lungs of rats (4); such proliferative changes may have imposed a functional handicap on the respiratory exchange and thus obscured a possible shock-protective effect which became evident in the passive transfer study.

The presence of antibody in serum from zymosan-treated rats and elevated complement levels in serum from glucan-treated rats

raises the possibility that immune factors may play a role in drum shock. This hypothesis is somewhat strengthened by the rough correlation between serum antibody content in zymosan-treated animals and the degree of passive transfer shock mortality.

Freedman has reported that following passive transfer of endotoxin-tolerant serum, the ability of a test dose of endotoxin to produce a pyrogenic (22) or lethal effect (16) was decreased; such protective actions could be attributed to RES stimulation (21) or an immunological mechanism (23). In our study, RES stimulation was not demonstrated 30 min following passive transfer of serum from zymosan- or glucan-treated rats, at least when compared to saline-injected controls. However, there was a suggestion that nonspecific components of normal serum and perhaps immune factors in serum from zymosan-injected animals enhanced the phagocytic capacity of recipient animals. Although the passive transfer of immune serum containing antibody specific for an endotoxin will increase the uptake of endotoxin by the RES of shocked dogs, endotoxin detoxification and survival rates in hemorrhagic shock are apparently not increased (24).

The role of complement in shock has not been extensively studied. Hemorrhagic shock does not evidently affect complement levels (25,26); however, such levels are significantly decreased following endotoxin shock (27). Recent studies have also reported that in canine endotoxin shock there was a prompt decrease in complement levels associated with increased plasma histamine (28); the latter was attributed to an immune mechanism. A possible relationship between elevated complement titers in serum from glucan-treated rats and the protective effect of this passively transferred serum in traumatic shock cannot be assessed with any certainty from our data.

The association of passive transfer shock protection with elevated complement levels in glucan-treated rats and with high antibody titers in zymosan-treated rats does however suggest that a further analysis of such immune mechanisms in shock along with studies of concomitant toxic effects of these two agents might be fruitful.

Summary. A single iv injection of zymosan (100 mg/ml) lowered the mortality of rats to drum shock 72 hours following injection; similar glucan injections (100 mg/ml) did not affect the shock mortality. Heat-stable complement fixing antibodies were present in serum from zymosan-treated rats; serum from glucan-treated rats had elevated complement titers. Intravenous passive transfer of whole serum from both zymosan- and glucan-injected rats to normal recipient rats lowered the mortality to subsequent drum shock in both groups of recipients; in neither recipient group was there any effect on carbon clearance when compared to saline-injected controls. There appears to be a correlation between serum antibody levels in zymosan-treated rats and passive-transfer shock mortality. The results suggest that in rats injected with zymosan or glucan, immune mechanisms may be concerned with their resistance to subsequent traumatic shock.

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1. McKenna, J. M. and Zweifach, B. W., Am. J. Physiol. 187, 263 (1956).

2. Zweifach, B. W., Benacerraf, B., and Thomas, L., J. Exptl. Med. 106, 403 (1957).

3. Filkins, J. P., Murphy, D. L., Doty, J. M., and Smith, J. J., Proc. Soc. Exptl. Biol. Med. 116, 757 (1964)

4. Filkins, J. P., Lubitz, J. M., and Smith, J. J., Angiology 15, 465 (1964).

5. Wissler, R. W., Fitch, F. W., and La Via, M. F., Ann. N. Y. Acad. Sci. 88, 134 (1960).

6. Halpern, B. N., J. Pharm. Pharmacol. 11, 321 (1959).

7. Rowley, D., Lancet 1, 232 (1955).

8. Blattberg, B., Proc. Soc. Exptl. Biol. Med. 92, 745 (1956).

9. Blattberg, B., Proc. Soc. Exptl. Biol. Med. 96, 81 (1957).

10. Pillemer, L. and Ross, O. A., Science 121, 732 (1955).

11. Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W., and Wardlaw, A. C., Science 120, 279 (1954).

12. Cutter, J. L., J. Immunol. 84, 416 (1960).

13. Wooles, W. R. and Di Luzio, N. R., Science 142, 1078 (1963).

14. Fine, J., "Shock, Pathogenesis and Therapy," Bock, K. D., ed., p. 25. Springer, Berlin, 1962.

15. Gilbert, R. P., "Shock Pathogenesis and

Therapy," Bock, K. D., ed., p. 40. Springer, Berlin, 1962.

16. Freedman, H. H., Proc. Soc. Exptl. Biol. Med. **102**, 504 (1959).

17. Biozzi, G., Benacerraf, B., and Halpern, B. N., Brit. J. Exptl. Pathol. **34**, 441 (1953).

18. Kabat, E. A. and Mayer, M. M., "Experimental Immunochemistry," p. 133. Thomas, Springfield, Illinois, 1961.

19. Chase, R. E., Master's thesis, Marquette Univ. Graduate School, Milwaukee, Wisconsin, 1967.

20. Nelson, R. A., Jr., J. Exptl. Med. **108**, 515 (1958).

21. Freedman, H. H., Ann. N. Y. Acad. Sci. **88**, 99 (1960).

22. Freedman, H. H., J. Exptl. Med. **111**, 453 (1960).

23. Greisman, S. E., Carozza, F. A., Jr., and Hills, J. D., J. Exptl. Med. **117**, 663 (1963).

24. Perreault, M., Schweinburg, F. B., Levenson, S., Israel, J., and Fine, J., Proc. Soc. Exptl. Biol. Med. **108**, 479 (1961).

25. Frank, E., Fine, J., and Pillemer, L., Proc. Soc. Exptl. Biol. Med. **89**, 223 (1955).

26. Levy, M. N., Blattberg, B., and Barlow, J. L., J. Lab. Clin. Med. **56**, 105 (1960).

27. Gilbert, V. C. and Braude, A. I., J. Exptl. Med. **116**, 477 (1962).

28. Spink, W. W., Davis, R. B., Potter, R., and Chartrand, S., J. Clin. Invest. **43**, 696 (1964).

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Effects of Progesterone, Testosterone, and Cortisol on Hypothalamic Prolactin-Inhibiting Factor and Pituitary Prolactin Content*† (32707)

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Injections of estrogen, progesterone, testosterone, or cortisol increased pituitary prolactin content and initiated mammary secretion in rats (1). Progesterone (2) and cortisol (3) retarded mammary involution in postpartum rats after litter removal. Administration of androgen (4) or progesterone (5) also induced pseudopregnancy in rats. These observations indicate that these 3 steroids can induce pituitary prolactin release in rats.

Nicoll and Meites (6) reported that estradiol can directly stimulate pituitary prolactin release *in vitro,* whereas progesterone, testosterone, and cortisol had no effect on pituitary prolactin release *in vitro*. They suggested that progesterone, testosterone, and cortisol may stimulate pituitary prolactin secretion *in vivo* by acting on the hypothalamus or through other mechanisms.

The primary influence of the central nervous system (CNS) on prolactin secretion is inhibitory, and the presence of a prolactin-inhibiting factor (PIF) in acid extracts of rat hypothalami has been demonstrated (7). Several agents such as estrogen (8), reserpine (9) epinephrine (10), acetylcholine (10), and Envoid (11) were shown to reduce PIF content in the rat hypothalamus and presumably thereby to promote prolactin secretion. The present study was undertaken to determine the effects of progesterone, testosterone, and cortisol on hypothalamic content of PIF, on pituitary prolactin concentration and on the mammary glands of ovariectomized female rats.

Materials and Methods. Mature female Sprague-Dawley rats (Spartan Animal Farms, Inc., Haslett, Mich.) averaging about 170–190 gm were used for all steroid injections. They were housed in a temperature ($75 \pm 1°F$) and light (14 hours/day) controlled room. The diet consisted of Wayne Lab Blox pellets (Allied Mills, Inc., Chicago, Ill.). Pituitary donors for incubations were adult mature male rats of the same strain, weighing 220–250 gm each.

All rats were ovariectomized and injected subcutaneously once daily with a steroid 1–2 weeks later. Progesterone (P) was given in a daily dose of 10 mg in 0.2 ml of corn

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