Sequential Changes in Reticuloendothelial System Function After Acute Hemorrhage[1] (36270)

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Evidence has accumulated to suggest that tolerance to various types of experimental shock and trauma may be associated with the functional capacity of the phagocytic elements of the reticuloendothelial system (RES) (see reviews 1–5, Refs.). For example, pretreatment of animals with various unrelated colloidal and chemical substances such as certain polysaccharides, choline chloride, denatured serum proteins, and certain triglycerides which stimulate RES phagocytic activity is in many instances associated with increased tolerance to shock (1, 2, 5–14); whereas materials which block or depress this system is, on the other hand, in many instances, associated with a predisposition to increased mortality (5–9, 11, 15, 16). In this regard, it is interesting to note that animals which are rendered tolerant to one form of shock are in many instances cross-tolerant to other forms of shock and trauma (5, 7, 8, 16–18). Such evidence has been used collectively, by Zweifach (1, 7–9), Zweifach *et al.* (7), Fine and his colleagues (19), Palmerio and Fine (20), and Altura and Hershey (5, 18), to suggest that the RES may represent the homeostatic system serving as a critical common pathway in the pathogenesis of, and host adaptation to, certain shock syndromes. Many of these chemicals and colloids are, however, not without certain toxic or pathologic effects on other bodily organ systems (3, 9, 14, 21, 22); a circumstance which, needless to say, makes such RES studies relevant to shock difficult to interpret. However, other evidence has been brought forth which indicates that when normal, unpretreated rats, rabbits, dogs, and mice are subjected to a variety of various types of experimental shock syndromes and trauma they exhibit early, profound impairment of RES phagocytic function (1, 4, 5, 16, 18, 23–25) which is sometimes followed by an enhancement (or stimulation) of RES function (4, 5, 16, 18, 23, 25). Furthermore, some of these latter experiments suggest that RES phagocytic function may undergo quantitative, sequential changes during the course of the shock syndrome (5, 16, 18, 23).

The object of this report is to present data which indicate that RES phagocytic function does, indeed, undergo sequential, quantitative changes in animals subjected to various degrees of acute hemorrhage through to recovery or death. Needless to say, such studies might provide quantitative data which, in conjunction with other hemodynamic and clinical measurements, could be used as an adjunct basis for developing simply derived diagnostic indices (at a tissue level) of the course of the shock syndrome.

Methods. Female rats of the Wistar strain, weighing 150 ± 20 g and anesthetized with im pentobarbital sodium (Nembutal, 30 mg/kg), were used for all of our studies. Different degrees of hemorrhagic shock were induced in different groups of paired animals. For example, control animals through to LD_{95} shock procedures were utilized in our experiments. Hemorrhage was induced by gradedly bleeding the anesthetized rats via a cannulated femoral artery over a 15–30 min

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period to a fixed volume of 3% body weight and withholding the blood from different groups of animals for different intervals of time. For example, an LD_{20} hemorrhage procedure resulted when the blood was withheld from the rats for a period of 1 hr. If the blood were withheld from the rats for 2 hr, 60% of the animals would die and so on. Sham procedures were identical with the hemorrhage procedure except that no blood was removed from the cannulated femoral artery. Where appropriate, animals were observed for survival for up to 5 days. (Animals that survived for 5 days from such hemorrhage procedures were found to live out their entire life spans.) All hemorrhaged animals were autopsied grossly at death for characteristic signs of shock. Blood pressure was carefully monitored in all experiments.

Phagocytic indices (or K values as they are termed) were determined from the rate of clearance of colloidal carbon, 4 mg/100 g of body weight, suspended in calf skin gelatin by using a slight modification (11) of the technique of Biozzi and co-workers (26). Precisely timed blood samples were obtained 2, 4, 8, 12, and 15 min after intravenous carbon injection, hemolyzed in 0.1% sodium carbonate and the carbon concentrations were measured photometrically at 675 mμ. Phagocytic indices were calculated:

$$K = (\log_{10} C_1 - \log_{10} C_2)/(t_2 - t_1)$$

where K is the phagocytic index, C_1 and C_2 are the colloidal carbon concentrations per 100 ml of blood at times t_1 and t_2. The means and standard errors of the means were calculated and statistically analyzed using Student's t test.

Results. Table I indicates the survival rates and mean K values for animals subjected to varying degrees of hemorrhage together with those of control and sham-operated animals. It is apparent from the K values that the greater the degree of hemorrhage (as defined here by the duration blood is withheld and the survival rate), the greater is the magnitude of early RES phagocytic depression. That is, the more severe the trauma, the lower the K value.

It is rather interesting to note that animals which survive these lethal hemorrhage procedures show, with time, progressively improved phagocytic indices (Table II), and furthermore such animals, usually with 24 hr, exhibited K values which are increased by more than 100% over normal control animals (Table II). These hyperfunctional RE systems usually returned to normal control levels by 96 hr.

TABLE I. Effects of Graded Hemorrhage on Survival and Carbon Clearance in Rats.[a]

Duration of time blood withheld (hr)	Survivors/ total	Survival (%)	Phagocytic index (K; mean ± SE)
Controls	35/35	100	0.046 ± 0.002 (35)[b]
Sham[c]	12/12	100	0.043 ± 0.005 (12)
1.0	17/22	77	0.020 ± 0.002[d] (16)
2.0	10/25	40	0.010 ± 0.002[d] (8)
2.5	5/25	20	0.004 ± 0.002[d] (8)
3.0	1/20	5	0.002 ± 0.003[d] (6)

[a] All of the hemorrhaged animals were bled 3.0% body weight. Test dose of colloidal carbon in calf skin gelatin = 4 mg/100 g of body wt. K values were obtained 1 hr after transfusion of shed blood. Survival determined at 5 days.

[b] Number of animals.

[c] Femoral arterial cannulation for 3 hr.

[d] Significantly different from control and sham-operated animal ($p < .01$).

In marked contrast to what happens in survivors, Table III indicates that animals which eventually die from acute hemorrhage exhibit depressed RE systems and thus fail to exhibit progressively improved K values (or hyperfunctional RE systems). Since 60% of the animals, that eventually died, usually succumbed in the first 12 hr after transfusion, we did not monitor RES phagocytic indices in this group of animals beyond the initial 12 hr.

Discussion. The present experiments strongly suggest that numerical RES phagocytic indices or K values correlate remarkably well with the intensity of the imposed acute hem-

TABLE II. Effects of Graded Hemorrhage on Carbon Clearance in Surviving Animals.[a]

Duration of time blood withheld (hr)	Phagocytic index (mean $K \pm$ SE) (hr):[b] 6	12	24
Controls	0.044 ± 0.005 (16)[c]	0.049 ± 0.006 (15)	0.041 ± 0.004 (16)
1.0	0.035 ± 0.008 (8)	0.060 ± 0.005 (10)	0.090 ± 0.003[d] (9)
2.0	0.020 ± 0.006[d] (9)	0.045 ± 0.005 (10)	0.080 ± 0.004[d] (10)
2.5	0.014 ± 0.004[d] (12)	0.035 ± 0.008 (8)	0.085 ± 0.005[d] (10)

[a] Carbon clearances determined only on animals which survived hemorrhage.
[b] Time elapsed after transfusion of shed blood.
[c] Number of animals.
[d] Significantly different from controls ($p < .01$).

orrhage episode and an animal's subsequent response towards either complete recovery or death. These experiments thus confirm and extend our earlier studies using a mild form of acute hemorrhage (27), trauma (18), and bowel ischemia shock (16, 23); these all being characterized by initial RES depression, followed by gradual recovery and development of RES hyperphagocytosis in both normal survivors and tolerant animals. Although qualitatively similar findings to ours (*i.e.*, RES depression followed by stimulation) have been recently reported by other laboratories for animals exposed to surgery, tourniquet shock, thermal and Noble-Collip trauma (4, 25, 28), thus essentially supporting our previous and present findings, the present report is, to our knowledge, the first demonstrations of: (i) this biphasic phenomenon in animals subjected to graded degrees of acute hemorrhage; and (ii) a correlation of K values to survival or death after shock.

The adaptive RES phagocytic changes, seen in the animals which survive various degrees of acute hemorrhage, probably result in more efficient handling (that is inactivation) of blood-borne tissue mediators, metabo-

TABLE III. Effects of Graded Hemorrhage on Carbon Clearance in Animals Which Fail to Survive the Insult.[a]

Duration of time blood withheld (hr)	Phagocytic index (mean $K \pm$ SE) (hr):[b] 3	6	12
Controls	0.042 ± 0.005 (18)[c]	0.049 ± 0.006 (15)	0.041 ± 0.004 (16)
1.0	0.023 ± 0.007[d] (8)	0.027 ± 0.006[d] (8)	0.025 ± 0.006[d] (6)
2.0	0.012 ± 0.006[e] (8)	0.011 ± 0.007[e] (8)	0.013 ± 0.009[e] (5)
2.5	0.004 ± 0.003[e] (8)	0.006 ± 0.005[e] (6)	0.005 ± 0.004[e] (4)

[a] Carbon clearances were evaluated here for only those animals which succumbed from the hemorrhage procedures. See Table I for mean K values obtained 1 hr after transfusion of shed blood.
[b] Time elapsed after transfusion of shed blood.
[c] Number of animals.
[d] Significantly different from controls: $p < .05$; [e] $p < .01$.

lites, and/or other noxious tissue products (1, 18). Although reduced blood flow *per se* may partially account for the early depression in RE phagocytic activity, this probably cannot account completely for the depression for several reasons which are reviewed elsewhere (4, 18, 28–30). Recent work by Schildt (29), Schildt and Bouveng (30), and Saba (28) strongly suggests that a depletion of plasma factors (*e.g.*, opsonins) observed after trauma and surgery, essential for RES phagocytosis, is probably the main cause of the early RES depression rather than an RES-depressing substance (31) or reduced blood flow. Such studies, however, have not as yet been undertaken in animals subjected to acute hemorrhage. As to what factor(s) is responsible for the marked, adaptive RES-induced stimulation seen in animals that either survive, and/or are tolerant to, the shock syndrome is, at the present time, speculative and has been discussed in detail elsewhere (18).

Within the present experimental framework, our data together with that reported previously by our group and others seem not only to support, but also strongly emphasize, the existence of a close, functional relationship between survival or death after shock and the status of the phagocytic elements of the RES. In addition, the present studies provide quantitative data which, in conjunction with previously reported information, could possibly be used as a basis for developing simply derived diagnostic indices, at a tissue level, of the course of the shock syndrome. Furthermore, other data indicates that vasoactive drugs, which are beneficial in the treatment of experimental bowel ischemic shock (*i.e.*, increase survival), also improve (enhance) the depressed RES function seen early in shock (5, 23). The latter findings when coupled with the present observations suggest that drugs exhibiting therapeutic value in shock should be evaluated not only for their effects on hemodynamic and metabolic parameters, but for their effects on RES function. Such data would add further support to the concept that RES function reflects the quality of host adaptive responses to shock and to shock therapy and that numerical RES phagocytic indices may be diagnostic and prognostic parameters of the shock syndrome. The recent appearance of several safe RES test substances (32-34) makes the feasibility of sequential RES determinations in man entirely practical.

Summary. Experiments were designed with rats to determine: (i) whether reticuloendothelial system (RES) phagocytic function undergoes quantitative, sequential changes after acute blood loss; and (ii) whether RES phagocytic indices (K values) are correlated with survival or death of the animals. The results indicate that: (i) RES phagocytic function does, indeed, undergo sequential, quantitative changes in animals subjected to various degrees of acute hemorrhagic shock. (ii) The greater the degree of hemorrhagic shock (as determined by mortality), the greater is the magnitude of early RES phagocytic depression. (iii) Animals which survive after various degrees of hemorrhagic shock not only show, with time, progressively improved RES phagocytic indices but exhibit hyperfunctional reticuloendothelial systems. (iv) Animals which succumb from these procedures fail to exhibit either progressively improved K values or hyperfunctional RE systems and thus continue to manifest RE systems which are markedly depressed up until death. Overall, these data could be used to suggest that numerical RES phagocytic indices may be diagnostic and prognostic parameters of the shock syndrome.

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