

Host Resistance to Bacteria in Hemorrhagic Shock V. Mobilization of Phagocytes.* (22217)

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We have given evidence that hemorrhagic shock reduces efficiency of the phagocytosis-promoting factor in serum(1), increases sensitivity to bacterial toxins(2), and decreases the Properdin activity of serum(3). This report deals with the effect of hemorrhagic shock on the capacity to mobilize phagocytes to the site of challenge.

Method. 50 cc sterile beef infusion broth was injected intraperitoneally into rabbits immediately after transfusion for hemorrhagic shock of 2 hours duration. The infusion was also given at the same time to normal rabbits of the same weight and litter. Six hours later, after white blood count was made, both groups were killed by exsanguination. The peritoneal cavity was opened immediately thereafter, and cellular exudate recovered.† For this purpose the abdominal incisions were fashioned to produce a trough into which gelatin-Locke's solution at 4°C was introduced and aspirated after a short interval of gentle but thorough prodding of the immersed viscera. Washing and aspiration was continued until a total of 500 ml of Locke's solution was used. The recovered fluid was then spun in refrigerated centrifuge, and the precipitate washed 3 times in Locke's solution to get rid of fibrin and fibrin clots. The number of cells recovered was counted, and the percent of different types of cells determined from a count of 200 cells in stained smears. To compare number of cells mobilized in normal and shocked animals in response to the stimulus of the injected broth, it was necessary to obtain control data on number of cells that can be obtained by the same peritoneal washing technic from peritoneal cavities of normal and shocked rabbits

of the same weight and litter, into which nothing has been injected. The shocked rabbits of this group were also killed 6 hours after transfusion.

Results. (Table I) A. *Control Data.* 1. *Normal rabbits—no intraperitoneal injection.* Number of cells recovered from 6 normal rabbits averaged 3.3 million (range 2.7-3.7 million). 65% of these were large mononuclear cells, 15% were lymphocytes and 20% were polymorphonuclear leucocytes.

2. *Shocked rabbits—no intraperitoneal injection.* Number of cells recovered from 6 shocked rabbits averaged 3.9 million (range 2.5-4.9 million). 75% of these were large mononuclear cells, 5% were lymphocytes, and 20% were polymorphonuclear leucocytes. The large mononuclear cells from both normal and shocked rabbits were elongated, somewhat flattened cells showing amoeboid activity. The fact that the number of cells in both groups was about equal, and that most of them were an apparently early form of the mature macrophage, suggests that they were for the most part traumatically desquamated peritoneal lining cells.

It is noteworthy that whereas no bacteria were observed in the cells from normal rabbits, ingested bacteria were seen occasionally in cells from 3 of the 6 shocked rabbits.

B. *Cellular Response 6 Hours After Intraperitoneal Injection of Beef Infusion Broth.* 1. *Normal rabbits.* The number of cells recovered from a series of 24 animals averaged 103 million (range 32-229 million). Of these 93% (range 85-98%) were polymorphonuclear leucocytes and 7% (range 15-2%) were macrophages, of which about half were mature, and the other half immature, *i.e.* like those recovered from the control animals. No bacteria were seen in stained smears of these cells. The white blood count in these rabbits

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† Sterile technic was employed in this and all further procedures where indicated.

TABLE I. Response of Normal Rabbits and of Rabbits Transfused after Two Hours Hemorrhagic Shock to Intraperitoneal Beef Infusion Broth Six Hours after Injection.

Intraper. stimulus	Cellular response in normal rabbits				Cellular response in shocked rabbits				Remarks
	Macro- phages	Polys	Lympho- cytes	Total in millions	% of	Macro- phages	Polys	Lympho- cytes	Total in millions
None	65*	20	15	3.3		75*	20	5	3.9
Beef infusion broth	74	93	—	103		75*	25	—	4.2

* Chiefly immature macrophages.

† 50% mature macrophages.

just before they were killed varied from 5800-6300 per cu mm.

2. *Shocked rabbits.* Twenty-four rabbits were prepared. Of these 6 died before the sixth hour following transfusion. Number of cells recovered from the 18 rabbits killed 6 hours after transfusion averaged 4.2 million (range 1.8-8.0 million if we except one from which 30 million were obtained). 75% of these (range 60-80%) were macrophages, most of which were immature, and 25% (range 40-20%) were polymorphonuclear cells. Just before these rabbits were killed their white blood cell counts were 3700-7600/cu mm.

In 8 of the 18 rabbits Gram positive and Gram negative bacilli and diplococci were found in considerable numbers.† There were about twice as many bacteria as cells per oil immersion field. Rather more than half of the bacteria were extracellular and the remainder intracellular. In addition *Coccidia* were observed in the exudate of 3 of the shocked rabbits.

Discussion. The considerable cellular response of the normal rabbit's peritoneal cavity to beef broth infusion demonstrates the irritant properties of this material. Since the number of cells that are recovered from the irritated peritoneal cavity of the shocked rabbit does not exceed the number recovered from the non-irritated cavity of the shocked rabbit, it is plain that shock severely impedes the mobilization of phagocytes, as Mahoney (4) and Miles and Niven(5) have demonstrated. That the cellular response should be poor *during* oligemic shock is not surprising, for the hemodynamic obstacle to mobilization is clear enough. But one would not expect this response in quantitative terms to be virtually zero during a 6 hour interval after restoration to a presumably normal hemodynamic status, in view of the fact that the white blood counts just before killing are about equal in normal and shocked rabbit. Since the normal animal's circulation delivers

† When clean but non-sterile technic was employed in harvesting the peritoneal exudate from normal rabbits, the only bacteria that we could recover were an occasional *B. subtilis*.

an average of 102 million polymorphonuclear leucocytes to the area of challenge, whereas the shocked animal's circulation delivers very few if any, the failure of these leucocytes in the shocked animal to enter area of challenge during post transfusion period appears to be due to some factor related to vascular permeability to these cells, rather than to a hemodynamic deficiency. This suggests that a mechanism such as the one recently described by Miles and Wilhelm(6), *i.e.* a cell permeability-promoting factor in serum, may have been inactivated by the shock process.

Another index of functional failure of the antibacterial defense system as a result of shock is inability of peritoneal lining cells and other precursors in the shocked animal to respond to the challenge for more macrophages, for the macrophages that are recovered are not increased in number, and of those recovered, most are still of the immature variety. This response contrasts with that of the normal rabbit in which the number of macrophages is about doubled, and about half of them are of the mature variety.

No bacteria were found in or outside the phagocytes in normal rabbit's exudate. But at least three varieties of bacteria, in a 2:1 proportion to cells, were found both in and outside the cells of the shocked rabbit.§ Since the stimulus to migration of bacteria from the intestine cannot be considered to be any less in the normal than in the shocked rabbit, the presence of bacteria in the shocked animal is further and unequivocal evidence of collapse

of the antibacterial mechanisms in shock.

Summary and conclusion. When the peritoneal cavity of the rabbit recovering from mild shock in response to transfusion is challenged by an irritant fluid, the cellular response as compared to that in a normal rabbit is extremely poor. The failure to mobilize polymorphonuclear leucocytes is, for the most part, not due to defective hemodynamics, but to a disturbance of vascular permeability to migrating leucocytes. The failure to supply additional macrophages at the site of the irritation appears to be due to a defect in the cellular processes involved in the conversion of their precursors. The presence of a substantial intracellular and extracellular bacterial population in the peritoneal fluid of the shocked animal, and the absence of bacteria in the peritoneal fluid of the unshocked animal, is further evidence of the loss of resistance to bacteria in the shocked animal, and of its persistence after restoration of a normal hemodynamic status.

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5. Miles, A. A., and Niven, J. S. F., *Brit. J. Exp. Path.*, 1950, v31, 73.

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§ Previous studies indicate that the source of these bacteria is the intestine and that route of invasion is by transmural migration(7).