his co-workers since malic oxidase was localized in the particulate fractions.

The only differences between strains which might be directly or indirectly concerned with virulence were found in the localization of cytochrome oxidase, isocitric dehydrogenase and alpha-ketoglutaric dehydrogenase. Cytochrome oxidase appeared to be soluble with the virulent H37Rv strain and particulate bound with the other strains tested. Isocitric and alpha-ketoglutaric dehydrogenases appeared to be particulate bound with the virulent H37Rv and its mutant H37RaN strain but soluble with the other strains tested. The significance of these differences must await further studies.

Summary. *Mycobacterium tuberculosis* var. *hominis* strains H37Rv, H37Ra and H37RaN; *Mycobacterium tuberculosis* var. *bovis* strain BCG-4; and *Mycobacterium smegmatis* were ground in a ball mill and fractionated according to the methods outlined earlier(2). Enzymes aconitase, fumarase, DPNH oxidase, catalase, cytochrome oxidase, isocitric, alpha-ketoglutaric, malic and succinic dehydrogenase were localized in one or more fractions using the two criteria established by Alexander and Wilson(12a, b). Cytochrome oxidase, DPNH oxidase and succinic dehydrogenase were found to be particulate bound by both criteria. However, the locus of other enzymes determined by one criterion did not always coincide with that determined by the second criterion. A modified criterion was then established and this was discussed. With the new criterion most of the enzymes of the tricarboxylic acid cycle and terminal electron transport system could be localized. The particles found within the various fractions were electron opaque and labile to electron bombardment. The presence of enzymatic activity, particularly those enzymatic activities concerned with energy yielding mechanisms lends support to the hypothesis that these particles are mitochondria.

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Received March 25, 1957. P.S.E.B.M., 1957, v95.

Host Resistance in Hemorrhagic Shock: VIII. Effect of Properdin on *in vitro* Function of Phagocytes.* (23246)

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Hemorrhagic shock in the dog lowers the Properdin titer in blood to or near zero within a few hours(1). Another effect of hemorrhagic shock is a fall in phagocytic and bacteriostatic indexes of phagocytes, which has been attributed to the presence of a leukotoxin in the plasma(2). Recently a lethal

* Aided by a grant from Natl. Heart Inst., N.I.H., Bethesda, Md., and by contract with Office of Surgeon General, U. S. Army.

TABLE I. Effect of Properdin on Functional Activity of Phagocytes in Normal and Shock Plasma.*

	Granulocytes				Macrophages			
	N.P.	N.P. + P.	S.P.	S.P. + P.	N.P.	N.P. + P.	S.P.	S.P. + P.
Phagocytic index	49 ± 10†	48 ± 8	21 ± 5	19 ± 7	44 ± 8	46 ± 8	23 ± 5	26 ± 5
Bacteriostatic index	69 ± 9	69 ± 11	20 ± 5	22 ± 7	41 ± 8	39 ± 4	21 ± 6	28 ± 8

* Each figure is mean of 4 or more experiments. † ± stand. dev.

N.P, normal plasma; P., Properdin; S.P., shock plasma.

toxin, in addition to a leukotoxin, has been identified in blood of hemorrhagic shock.† Whether either toxin is a bacterial endotoxin, or a polysaccharide derived from host's tissues(3), it is possible that the fall in titer of Properdin in shock plasma might be due to binding to toxin. Although the depression of the phagocytic and bacteriostatic activity of phagocytes is presumably caused by the toxin, it is possible that it might be caused by a deficiency of Properdin. In either case one might expect to restore the function of these cells to normal by providing an excess of Properdin. This was investigated by *in vitro* studies of the effect of added Properdin upon phagocytic and bacteriostatic activity of phagocytes in plasma of normal and shocked animals.

Method. Macrophages and granulocytes were harvested from peritoneal cavities of normal rabbits, and from peritoneal cavities of rabbits 6 hours after intraperitoneal injection of beef infusion broth, by a method described elsewhere(4). Plasma was obtained from normal rabbits, and from rabbits in advanced hemorrhagic shock following failure to respond to transfusion. The following mixtures of plasma (0.2 ml) and cells (10 million cells in 0.1 ml of gelatin-Locke's solution) were prepared in duplicate: granulocytes in normal plasma; granulocytes in shock plasma; macrophages in normal plasma; and macrophages in shock plasma. To each mixture 0.01 ml of a 5 hour culture of Friedlander's bacillus was added, and the phagocytic and bacteriostatic activity of the cells determined by a technic described elsewhere(2). The same tests were run simultaneously in duplicate, using plasma fortified with Properdin‡ added to yield a concentration of 32 u/ml.

Results. Table I lists the phagocytic and bacteriostatic indexes of granulocytes and macrophages in normal and shock plasma, and in the same plasmas with Properdin added. In accordance with Pillemer's findings,§ Properdin had no effect on phagocytosis. This is true for both types of cell, and in both normal and shock plasma. The same results apply to bacteriostasis.

To eliminate the possibility that an excess of Properdin might inhibit a salutary effect of this substance, several determinations were performed using only 8 units of Properdin/ml of plasma. The results were the same as with the higher concentration of Properdin. Several experiments were done with another test organism (*E. coli*). In this case, too, Properdin did not alter the phagocytic or bacteriostatic activity of the cells.

The depression in phagocytic and bacteriostatic activity of the phagocytes by shock plasma can be eliminated by diluting the shock plasma with normal plasma. The data in Table II show that Properdin added to progressive dilutions of shock plasma with normal plasma does not materially influence the phagocytic or bacteriostatic index of the cells.

Conclusion. The phagocytic and bacteriostatic activity of phagocytes *in vitro* is not influenced by the titer of Properdin in plasma

† Schweinburg, F. B., Shapiro, P., Fine, E., and Fine, J., PROC. SOC. EXP. BIOL. AND MED., in press.

‡ Properdin, complement and magnesium ions constitute the components of the Properdin system(5). It has been previously demonstrated that complement does not fall in hemorrhagic shock(6). Although no magnesium determinations were performed, significant electrolyte imbalance does not develop in simple hemorrhagic shock produced by the technic we employ.

§ Personal communication.

TABLE II. Effect of Normal Serum with and without Properdin on Functional Activity of Granulocytes in Shock Plasma.

N.P. added to S.P.		Phagocytic index (Mean of 4 exp.)	Bacteriostatic index (Mean of 4 exp.)
.00	.2	15 ± 3	24 ± 7
.01	.19	15 ± 3	29 ± 8
.02	.18	18 ± 6	31 ± 14
.04	.16	27 ± 3	31 ± 14
.08	.12	39 ± 8	43 ± 15
.16	.04	44 ± 8	68 ± 19
.20	.00	49 ± 7	68 ± 12
N.P. + P. added to	S.P.	Phagocytic index (Mean of 4 exp.)	Bacteriostatic index (Mean of 4 exp.)
.00	.2	15 ± 3	24 ± 7
.01	.19	16 ± 4	31 ± 9
.02	.18	21 ± 2	33 ± 8
.04	.16	31 ± 8	51 ± 9
.08	.12	39 ± 5	73 ± 8
.16	.04	45 ± 8	69 ± 9
.20	.00	49 ± 7	68 ± 12

of normal or shocked animals.

We are grateful to Dr. Louis Pillemer for the Properdin, which was stored at -20°C until immediately before use.

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Received March 27, 1957. P.S.E.B.M., 1957, v95.

Failure to Transmit Radiation-Induced Early Hypotension in Rabbits Connected Through Controlled Cross-Transfusion.* (23247)

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In rabbits, whole-body x-irradiation with doses above 50 r induces a blood pressure fall that becomes evident within 2 hours after exposure(1,2). Some experimental findings suggest that a similar hypotensive reaction may occur in a normal test animal after injection of blood originating from an irradiated rabbit(1). Is the blood pressure drop really caused by a blood-borne chemical factor? A decisive answer may be expected from continuous cross-transfusion studies. This type of experiment requires a high rate of blood exchange throughout a period of several hours, and conservation of a stable mean blood volume in each of the connected animals, even though a difference in blood pressure may develop. The present report describes a blood pump that fulfills the requirements and some results obtained with the equipment.

Methods. General procedure. With the arrangement depicted in Fig. 1, cross-transfusion was started at 30 min prior to irradiation, continued throughout the exposure time of 30 min. and concluded approximately 3 hours after irradiation. Then the blood volume of each rabbit was determined by the Evans blue method and the body weight remeasured. During transfusion the sequence of events was as follows: (I) withdrawal of blood from first animal, (II) change of valve position, (III) injection into second animal, (IV) withdrawal of blood from second animal, (V) change of valve position, (VI) injection into first animal; thereafter the next cycle started again with (I). In the present experiments motor speed was so regulated as to furnish three complete cycles every minute. Dead space in polyethylene tubing and valve amounted to 1/40 cc; therefore, a stroke vol-

* The author is indebted to H. A. Jaeger who constructed the cross-transfusion pump in the USAF School of Aviation Medicine.