factor limiting stimulation. This same observation, however, was made on only 1 of 5 animals, kept at 98°F, in the present experiment. In addition, it is known that marrow in the hypophysectomized rat is less sensitive to various stimuli, *i.e.*, anoxia(4), and bleeding(5-7); therefore, until such time as hypophysectomized rats are maintained at temperatures equal to normal or even higher than normal, the lowered body temperature cannot be eliminated as possibly having had an influence on the results.

Huggins and Blocksom also showed that anemia induced by repeated bleeding or splenectomy of animals infected with Bartonella did not stimulate hematopoiesis in the tail bones of normal rats, but the accompanying anemia resulted in a more extensive hematopoietic response in bones transposed to the abdominal cavity. The anemia of hypophysectomy does not have this stimulatory effect.

It is of interest to note that in the present experiment hypophysectomy resulted in an inhibition of both erythropoiesis and myelopoiesis. This is in contrast to the results obtained by Meineke and Crafts(3) where, in femur marrow, hypophysectomy induced a depression in erythropoiesis only; there was no effect on myelopoiesis.

It is possible that hypophysectomy has removed some hormone or caused some change in the animal which is necessary for the thermal stimulus to be effective. This could be the direct effect of removal of a single hormone (such as growth hormone), a combination of hormones, or might be the result of a secondary effect on metabolism. Reparative hormonal therapy under conditions outlined in this experiment might be of value in providing information as to the mechanism involved in the initiation of hematopoiesis.

Summary. Thirty-seven of 41 normal female rats, in which the ends of the tails were transposed into the abdominal cavity, showed a stimulation of hematopoiesis in the intra-abdominal vertebrae. Developing cells of all 3 types—erythroid, myeloid and megakaryocytes—were observed in these animals. In similarly treated hypophysectomized female rats only 1 of 16 animals showed any indication of response. The experiment provides another example of the depressed hematopoietic potential of the hypophysectomized rat.

1. Huggins, C., and Blocksom, B. H., Jr., *J. Exp. Med.*, 1936, v64, 253.

2. Huggins, C., Blocksom, B. H., Jr., and Noonan, W. J., *Am. J. Physiol.*, 1936, v115, 395.

3. Meineke, H. A., and Crafts, R. C., *Anat. Rec.*, 1956, v124, 47.

4. Feigin, W. M., and Gordon, A. S., *Endocrinology*, 1950, v47, 364.

5. Querido, A., and Overbeek, G. A., *Arch. Internat. Pharmacodyn. et de Therp.*, 1939, v61, 475.

6. Finkelstein, G., Gordon, A. S., and Charipper, H. A., *Endocrinology*, 1944, v35, 267.

7. Silbergleit, A., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 170.

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Resistance to Bacteria in Hemorrhagic Shock. VII. Demonstration of Leucotoxin in Plasma of Shocked Rabbit.* (22794)

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In the first of this series of studies it was shown that the phagocytic index of granulocytes from the peritoneal cavity of the normal dog, immersed in plasma from the dog in hemorrhagic shock, falls more and more the longer the dog has been in shock when the

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test plasma is taken(1). This was attributed to a progressive decline in the potency of phagocytosis-promoting factors in the plasma. But it would have been just as valid to interpret the data as signifying a progressive rise in the potency of a phagocytosis-inhibiting factor in the plasma. In the present communication we report evidence that the plasma of the shocked rabbit contains a substance that inflicts functional and morphologic damage upon normal phagocytes *in vitro*.

Method. The unchallenged peritoneal cavity of a normal or shocked rabbit, when washed with iced gelatin Locke's solution, yields a cell suspension containing 70% macrophages. Thus one can obtain and compare these cells from the normal and shocked rabbit. But a comparison of granulocytes is not possible, because they appear in large numbers and as the predominant cell after several hours in response to an irritant injected into the peritoneal cavity of the normal rabbit, but they do not appear in significant numbers in response to the same stimulus in the shocked rabbit, either during shock or for several hours after an effective transfusion. The reason for the failure of granulocytes to leave the circulation in the shocked rabbit may be because a severe granulocytopenia develops soon after shock is induced and persists for some hours, even after an effective transfusion. For the same reason the blood is an unpromising source of a sufficient supply of these cells. We have, therefore, obtained data on the effect of plasma from normal and shocked rabbits upon the morphology and function of (a), macrophages from the peritoneal cavity of the normal and shocked rabbit and of (b), granulocytes from the peritoneal cavity of the normal, but not of the shocked rabbit. By a technic described elsewhere(2) macrophages were obtained from the peritoneal cavities of normal and shocked rabbits, and granulocytes were obtained from the peritoneal cavities of normal rabbits. They were promptly harvested by centrifugation for 20 minutes at 4°C, washed 3 times in iced gelatin Locke's (g-L) solution, and resuspended in a final concentration of 10 million cells per 0.1 ml of the g-L solution. Plasma was obtained from normal rabbits and from rabbits which were either in advanced shock (irreversible to transfusion), or in shock of 2 hours duration (reversible shock). In the latter it was taken at the end of the 2 hours, just before transfusion, or 4 hours after the transfusion. The g-L solution and the plasma were heparinized (4 mg/100 ml). *For study of comparative morphology of cells in normal and shock plasma,* 0.5 ml of plasma was added to 0.1 ml of cell suspension and the mixture incubated for one hour at 37°C. Smears of the mixture were then made and stained with methylene blue (for nuclei) or Wright's stain (for cytoplasm). All preparations were made in duplicate. Granulocytes were classified as mildly injured when the nucleus showed a change from the multilobular to an oval or crescent shape and a shift to the periphery of the cytoplasm. More severe injury consisted of distortion of the cytoplasm, with blurring of detail and loss of granularity, and conversion of the nucleus to a crescent shaped amorphous mass displaced to the periphery of the cytoplasm. Such cells resemble L. E. cells. The percent of injured cells was determined from counts of 200 cells in each preparation. The mildly injured cells were counted separately from the severely injured ones. Less than 2% of granulocytes freshly garnered from the peritoneal cavity and suspended in the g-L solution show mild or severe injury. After they are allowed to incubate in the g-L solution or in normal plasma for one hour at 37°C, not more than 5% show such injury. *To determine reversibility of morphologic injury* to granulocytes, the shock plasma and cell mixture was incubated at 37°C for one hour, then washed 3 x in iced g-L solution, and then resuspended in the g-L solution or in normal plasma, and again incubated for one hour at 37°C. Smears were made after the first and second incubation, and any change in the percentage of injured cells noted. *For study of comparative phagocytic and bacteriostatic power of phagocytes* in normal and shocked plasma the following technic, adapted from Wood and Smith(3), was employed: 0.1 ml of cell suspension was added to 0.01 ml of a 5 hour culture of Friedlander's bacillus (about one million bacteria). To this mixture 0.1 ml of normal or shock

plasma was added. (Since storage for some days at −20°C was found not to change the effect of plasma on the behaviour of phagocytes, the test plasma was either fresh or stored.) Aliquots (0.015 ml) of this mixture were incubated on 1 cm squares of filter paper in a moist Petri dish at 37°C for one hour. One square was then smeared on a glass slide, and the latter stained with methylene blue. The percentage of bacteria ingested, and the percentage of cells with ingested bacteria were determined by counts of the intracellular and extracellular bacteria in fields containing a total of 200 cells. The remaining filter paper preparations were pooled and washed until clean with 0.5 to 1.0 ml of iced g-L solution. The washings were transferred to a centrifuge tube and spun for 5 minutes at 800 rpm in a refrigerated centrifuge to precipitate the cells. The supernatant was decanted and spun for 15 minutes at 2500 rpm to harvest the uningested bacteria. The cell precipitate was triply washed in iced g-L solution to remove uningested adherent bacteria. The washed cell precipitate, to which 1 ml of iced g-L solution was added, was then transferred to a mortar containing sterile sand, and ground for 15 seconds in order to rupture the cells and release the ingested bacteria. The mixture was transferred with added iced g-L solution to a centrifuge tube, and spun for 5 minutes at 800 rpm to remove the sand and cellular debris. The ingested bacteria were now free in the supernatant, which was decanted and spun at 4°C for 15 minutes at 2500 rpm to harvest these bacteria. Separate "agar smears" were made from the precipitates of uningested and of ingested bacteria. These smears were prepared by spreading a minute sample of each precipitate on a coverslip previously coated with a layer of melted agar, then covering the smear with another layer of melted agar. The coverslips were then transferred to a covered Petri dish and incubated for 2 hours at 37°C. They were then stained with methylene blue, inverted, mounted on a glass slide and sealed with paraffin. Counts were made of the number of single bacteria (dead†) and of colonies (live) and the percent of dead bacteria determined.

TABLE I. % of Granulocytes Showing Morphologic Injury after Incubation for One Hour at 37°C in Shock Plasma* in 6 Experiments.

Reversible shock plasma, no transfusion			Reversible shock plasma, 4-6 hr after transfusion			Irreversible shock plasma, no transfusion		
M†	S‡	T§	M	S	T	M	S	T
20	50	70	15	15	30	27	57	84
16	14	30	40	15	55	20	14	34
18	15	33	20	10	30	18	14	32
23	34	57	30	30	60	18	18	36
			30	25	55	31	37	68
			20	60	80			

* Effect of normal plasma was determined in every case simultaneously with effect of shock plasma on the same cell suspension. Because normal plasma did not produce damage to more than 5% of the cells in any case, data for normal plasma are omitted.

† M—Mild injury. ‡ S—Severe injury ("L.E." cells). § T = M + S.

Some 6 determinations of the phagocytic and of the bacteriostatic activity were made in duplicate in each of the following categories: 1, normal macrophages in normal plasma; 2, normal macrophages in shock plasma; 3, macrophages from shocked rabbits in normal plasma; 4, macrophages from shocked rabbits in shock plasma; 5, granulocytes from normal rabbits in normal plasma; and 6, granulocytes from normal rabbits in shock plasma.

Results. A. *Effect of shock plasma on morphology of phagocytes.* (Fig. 1 and 2). 1. *Macrophages.* These cells appear normal when freshly harvested from the unchallenged peritoneal cavity of the shocked as well as of the normal rabbit. No change from normal was observed after standing for one hour at 37°C in either g-L solution or in normal plasma. But after standing at 37°C for one hour in plasma from rabbits in advanced shock some 20% showed damage consisting of any or all of the following changes: Small and large vacuoles in the cytoplasm, loss of definition of the cell wall, pyknosis of the nucleus, or blurring of its membrane. 2. *Granulocytes* (Table I). Not more than 1-2% of these cells show abnormal morphology when examined immediately after harvesting from

† Single bacteria might be inhibited rather than dead. But the general significance of the observation is valid, whichever is the case.

the peritoneal cavity. After these cells are immersed in g-L solution or normal plasma for one hour at 37°C, the percentage of damaged cells does not exceed 5%. Table I lists the data on plasma from reversibly shocked and irreversibly shocked rabbits. Even if one

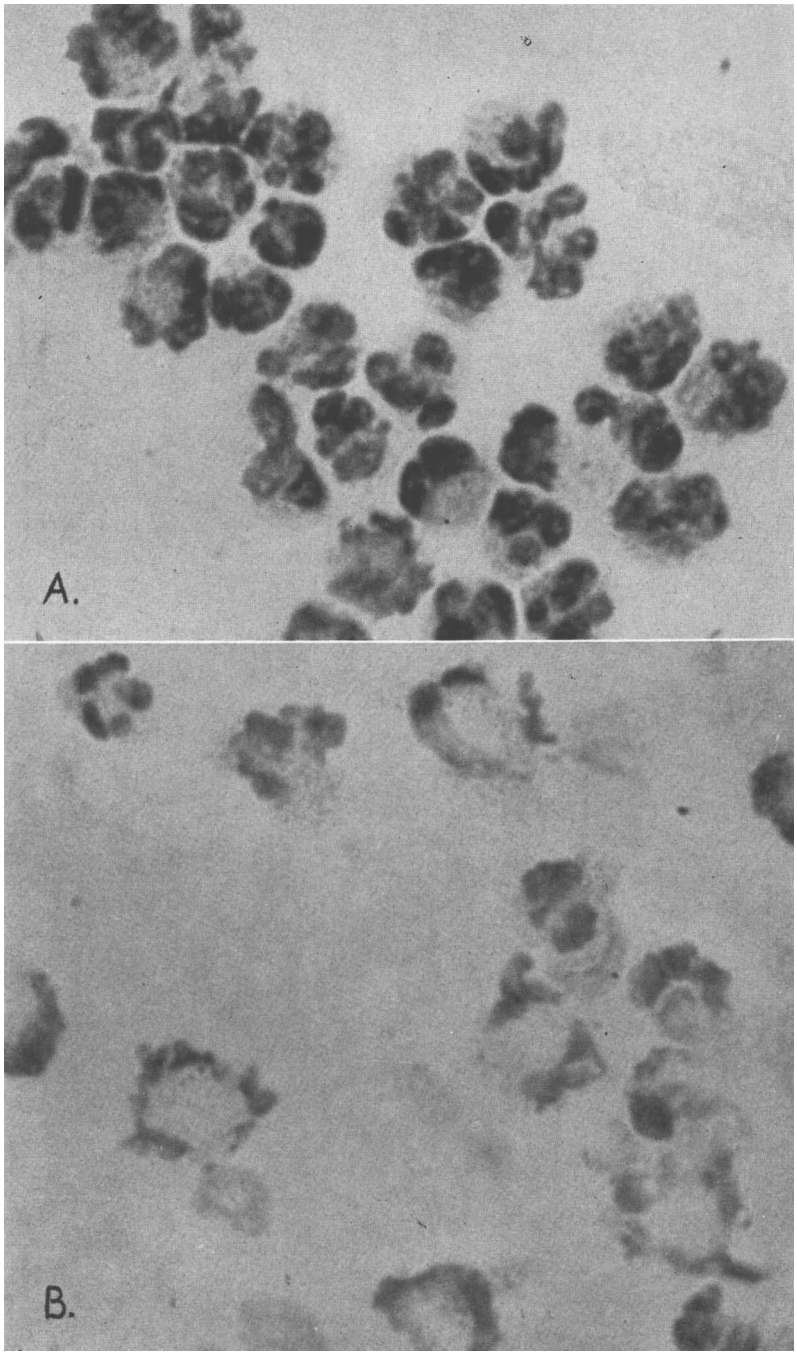

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FIG. 1. A. Granulocytes from peritoneal cavity of normal rabbit washed in iced gelatin–Locke's solution, and then suspended in fresh plasma from a normal rabbit. B. Same as A, except that plasma is from a rabbit in hemorrhagic shock of 2 hr duration.

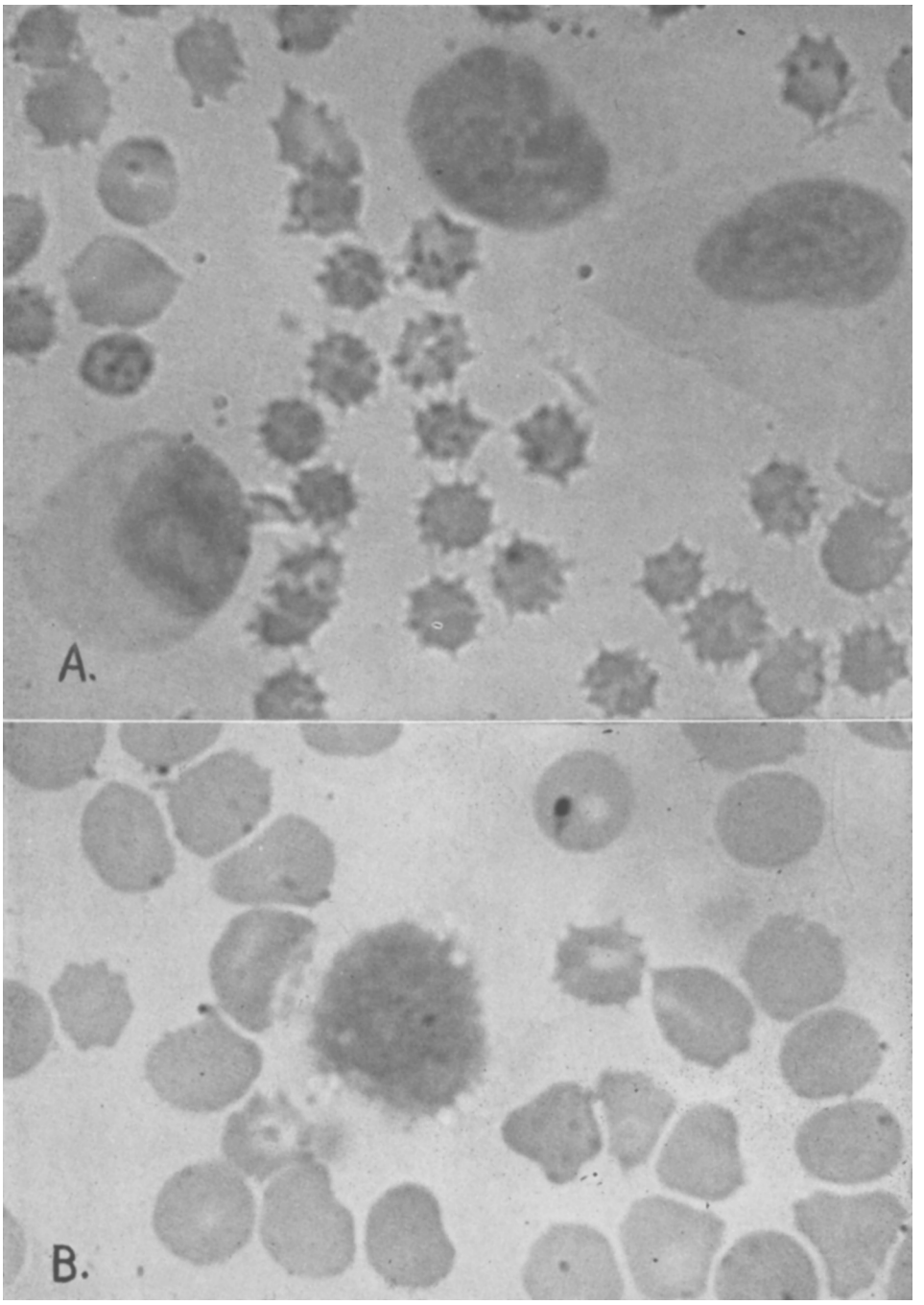

FIG. 2. A. Macrophages from peritoneal cavity of normal rabbit—washed in iced gelatin—Locke's solution and then suspended in fresh plasma from normal rabbit. B. Same as A except that plasma is from a rabbit in hemorrhagic shock of 2 hr duration.

TABLE II. % of Normal Granulocytes Showing Injury after Incubation for One Hour at 37°C in Reversible Shock Plasma followed by Incubation for One Hour in g-L Solution or Normal Plasma. 6 experiments.

Reversible shock plasma, 4-6 hr after transfusion			Gelatin, Locke's sol.			Normal plasma		
M*	S†	T‡	M	S	T	M	S	T
15	15	30	5	3	8	5	0	5
40	15	55	6	5	11	5	3	8
20	10	30	2	2	4	3	3	6
30	30	60	5	13	18	11	2	13
30	25	55	5	5	10	8	4	10
20	60	80	8	18	26	5	15	20

* M—Mild injury.
† S—Severe injury ("L.E." cells).
‡ T—M + S.

is inclined to accept the "L.E." cells only as sufficiently significant evidence of morphologic injury, there is no doubt that shock plasma is distinguishable from normal plasma by the presence in shock plasma of a cell damaging factor.

Reversibility of morphologic injury. That the morphologic damage is reversible is shown in Table II, which compares the percentage of cells damaged by irreversible shock plasma with the percentage of the same cells washed free of this plasma and subsequently exposed for one hour at 37°C to g-L solution or to normal plasma. The technic of the shock experiment, as we perform it(4), allows continuous exchange between the blood in the circulation of the animal and the blood lost by the animal into an elevated reservoir. It is conceivable that the blood, which stands in the reservoir at room temperature for several hours, may generate a toxic factor that is factitious, and that such a toxic factor, rather than one developing in the animal, accounts for the effect of shock plasma upon the integrity of the phagocytes. That this is not so is indicated by the data in Tables III and IV, which show (1), that the granulocytopenia which develops in shock does not affect the differential count of the blood in the reservoir (Table III); (2), that the number of granulocytes in the latter remains near normal (Table III); (3), that the percent of normal granulocytes damaged by the animal's plasma after 2 hours of shock is substantially

TABLE III. Comparison of Differential Leucocyte Count in Circulating Blood after 2 Hr of Hemorrhagic Shock with Differential Leucocyte Count in Blood in Elevated Reservoir.

Exp. No.	Type of cell	In circulating blood before shock	In circulating blood after 2 hr of shock	In elevated reservoir
1	P*	67	41	73
	L†	27	55	23
	M‡	6	4	4
2	P	67	31	68
	L	30	69	29
	M	3	0	3
3	P	61	19	45
	L	38	79	54
	M	1	2	1
4	P	51	34	60
	L	47	66	39
	M	2	0	1

* P—Polymorphonuclear leucocytes.
† L—Lymphocytes.
‡ M—Mononuclear cells.

greater than the percent damaged by the plasma in the reservoir (Table IVA); and (4) that the phagocytic and bacteriostatic potency of normal granulocytes immersed in plasma from the reservoir after 2 hours of shock is distinctly greater than that of the

TABLE IV-A. Comparison of % of Mildly and Severely Damaged Granulocytes Immersed in Plasma from Circulating Blood and in Plasma from Blood in Elevated Reservoir after 2 Hr of Hemorrhagic Shock. 4 experiments.

Plasma from circulating blood			Plasma from blood in reservoir		
M*	S†	T‡	M	S	T
18	15	33	20	4	24
23	34	57	6	7	13
20	50	70	3	4	7
16	14	30	10	3	13

* M—Mild injury.
† S—Severe injury.
‡ T—M + S.

TABLE IV-B. Comparison of Phagocytic and Bacteriostatic Potency of Normal Granulocytes Immersed in Plasma from Blood in Reservoir after 2 Hr and in Plasma from Circulating Blood after 2 Hr in Shock. 4 experiments.

Phagocytic index		Bacteriostatic index	
SP*	RP†	SP	RP
10	45	30	49
18	27	15	44
14	50	19	50
23	40	32	76

* SP—Reversible shock plasma.
† RP—Plasma from blood in reservoir.

TABLE V. Effect of Irreversible Shock Plasma on Percentage of Bacteria Ingested by Phagocytes.*

Normal macrophages† in			Shock macrophages‡ in			Normal granulocytes§ in		
		S.P.**			S.P.			S.P.
N.P.‖	S.P.¶	N.P.	N.P.	S.P.	N.P.	N.P.	S.P.	N.P.
35	18	.51	34	20	.60	53	26	.49
44	31	.70	37	30	.80	42	21	.50
33	20	.60	37	16	.43	50	32	.64
31	16	.51	30	17	.56	31	19	.61
55	34	.61	50	21	.42	60	27	.45
—	—	.59	—	—	.56	—	—	.54

* Phagocytic activity = % of bacteria in field of 200 intracellular phagocytes. Figures are in %.

† Macrophages harvested from unchallenged peritoneal cavity of normal rabbit immediately after killing.

‡ Macrophages harvested from unchallenged peritoneal cavity of rabbit killed 4 hr after transfusion for shock of 2 hr duration.

§ Granulocytes from peritoneal cavity of normal rabbit killed 6 hr after inj. of beef infusion broth.

‖ N.P.—Normal plasma. ¶ S.P.—Plasma from rabbit shortly before death from hemorrhagic shock, not responsive to transfusion. ** $\frac{\text{S.P. Phagocytic index in shock plasma}}{\text{N.P. Phagocytic index in normal plasma}}$.

same cells immersed in the plasma from the circulating blood taken at the same time.

B. *Effect of shock plasma on phagocytosis. Macrophages.* From Table V one can conclude that: (1) there is no significant difference between the percent of bacteria ingested by macrophages taken from the normal rabbit and the percent of bacteria ingested by macrophages from the rabbit dying of irreversible shock. (2) The percent of bacteria ingested by macrophages from the normal and shocked rabbit and by normal granulocytes is depressed to about the same extent by shock plasma, *i.e.* by somewhat less than 50%. (3) The deficient phagocytosis is due to a toxic factor in the plasma of the shocked animal. Table VI shows that the shock plasma reduces the percentage of cells with ingested bacteria. We have no data to show whether the reduced phagocytic index is due to the total suppression of phagocytosis by some cells or to a reduction in the average number of bacteria ingested per cell. To determine whether the amount of inhibiting factor depends upon the duration of shock, we have compared the degree of depression of the phagocytic indexes in the various types of shock plasma in Table VII. No significant difference between the toxicity of the plasma of reversible and irreversible shock appears in the data except those on the activity of granulocytes, which, though suggestive, are too meager to permit a definitive inference.

TABLE VII. Comparative Depression of Phagocytic Activity of Phagocytes by Plasma from Rabbits in Reversible and Irreversible Shock.*

	Normal macrophages in		Shock macrophages in		Normal granulocytes in	
	R'SP†	ISP‡	R'SP	ISP	RSP§	ISP
	.51	.56	.60	.50	.66	.49
	.70	.70	.80	.63	.74	.50
	.60	.41	.43	.46	.70	.64
	.51	.71	.56	.60	.62	.61
	.61	.59	.42	.65	—	.45
Avg	.60	.60	.56	.57	.68	.54

* Figures in each case represent ratio of phagocytic index of cells in shock plasma to phagocytic index in normal plasma.

† R'SP—Plasma taken 4 hr after transfusion for 2 hr of shock.

‡ ISP—Plasma taken from rabbits before death from advanced shock. No transfusion.

§ RSP—Plasma taken after 2 hr of shock. No transfusion.

C. *Effect of shock plasma on bacteriostatic potency of phagocytes.* Table VIII lists the percentage of ingested bacteria rendered bacteriostatic by phagocytes after immersion for one hour at 37°C in normal plasma, and in plasma from reversibly and irreversibly shocked rabbits. The depression of the bacteriostatic potency of these cells by the various shock plasmas is expressed in terms of the

TABLE VI. Effect of Shock Plasma on Percentage of Cells Containing Bacteria.

Normal macrophages in			Normal macrophages in			Shock macrophages in			Shock macrophages in			Normal granulocytes in			Normal granulocytes in		
		R'SP			ISP			R'SP			ISP			RSP			ISP
NP*	R'SP‡	NP	NP	ISP§	NP	NP	R'SP	NP	NP	ISP	NP	NP	RSP†	NP	NP	ISP	NP
40	38	.95	22	16	.73	52	20	.40	20	16	.80	30	18	.60	41	20	.49
30	19	.63	23	16	.69	28	20	.71	23	14	.61	40	30	.75	30	18	.60
33	25	.75	41	33	.80	34	18	.71	40	18	.45	33	31	.94	39	31	.80
25	25	1.00	30	27	.90	20	18	.53	28	25	.89	24	18	.75	20	18	.90
33	20	.60	24	18	.75	18	20	.90	27	18	.66	—	—	—	41	29	.70
34	25	.74	—	—	—	30	25	1.11	—	—	—	—	—	—	—	—	—
Avg		.78			.77			.73			.68			.76			.70

* NP—Normal plasma. † RSP—Plasma taken after 2 hr of shock. No transfusion. ‡ R'SP—Plasma taken 4 hr after transfusion for 2 hr of shock. § ISP—Plasma taken from rabbits before death from advanced shock. No transfusion.

TABLE VIII. Effect of Shock Plasma on Bacteriostatic Potency of Phagocytes.

Normal macrophages in						Shock macrophages in						Normal granulocytes in								
		R'SP			ISP			R'SP			ISP			RSP			R'SP			ISP
NP*	R'SP†	NP	NP	ISP‡	NP	NP	R'SP	NP	NP	ISP	NP	NP	RSP§	NP	NP	R'SP	NP	NP	ISP	NP
49	29	.59	37	20	.54	50	30	.66	40	18	.45	68	30	.44	60	28	.47	55	27	.49
40	30	.75	—	—	—	46	25	.61	41	25	.61	51	15	.30	67	31	.46	71	30	.42
51	25	.49	50	27	.54	50	22	.44	37	20	.54	46	19	.41	57	29	.57	64	24	.37
35	25	.71	40	29	.73	30	25	.83	22	15	.68	80	32	.40	48	20	.42	51	18	.35
38	29	1.76	25	15	.60	40	32	.80	40	20	.50	—	—	—	—	—	—	68	31	.46
24	20	.59	38	21	.55	28	20	.63	—	—	—	—	—	—	—	—	—	—	—	—
Avg		.65			.59			.65			.56			.39			.47			.51

* NP—Normal plasma. † R'SP—Plasma taken 4 hr after transfusion for 2 hr of shock. ‡ ISP—Plasma taken from rabbits before death from advanced shock. No transfusion. § RSP—Plasma taken after 2 hr of shock. No transfusion.

ratio of the percentage of ingested bacteria that did not multiply when exposed to shock plasma to the percentage of ingested bacteria that did not multiply when exposed to normal plasma. These data demonstrate a considerable degree of damage to the bacteriostatic potency of phagocytes by shock plasma. They also demonstrate that the injury is about the same whether the shock is reversible or irreversible, and that the toxin in the plasma is still as active 4 hours after transfusion for 2 hours of shock as it is immediately after 2 hours of shock. Furthermore, it appears that the leucotoxin reduces the bacteriostatic potency of granulocytes to a greater degree than of macrophages.

Discussion. From the foregoing data we conclude that a leucotoxin is present in the plasma of hemorrhagic shock. This conclusion rests chiefly upon the evidence relating to the functional behaviour of the phagocytes. Since we have no data on the functional capacity of the morphologically damaged phagocytes, we cannot say whether the morphologic injury bears any relation to the functional injury. Nor can we say that the leucotoxin responsible for the functional injury is the substance causing the morphologic injury.

What is the source of the leucotoxin? The evidence given is sufficient to exclude a substance that might be produced in consequence of the special conditions of our shock experiment. The phagocytes and the plasma in the relatively stagnant blood in the reservoir have been found to be comparable in behaviour to the phagocytes and the plasma from the blood of the normal rabbit. And the transfusion has been excluded as a factor in the toxicity of the plasma of the animal recovering from shock, and of the animal dying of shock after a transfusion.

Is the leucotoxin an endotoxin? Endotoxins given intravenously are known to produce granulocytopenia. We have data to be published which show that endotoxins, added to a mixture of normal plasma and normal granulocytes in concentrations equivalent to those in circulating blood after injecting a sublethal dose, produce morphologic damage which is virtually the same as that produced by plasma from the shocked animal. Endotoxins also damage the antibacterial defense mechanisms and produce typical irreversible peripheral vascular collapse(5). Elsewhere(6) we have put forward the view that hemorrhagic shock weakens the antibacterial defense, in consequence of which unfettered bacterial activity eventually inflicts irreversible damage to the peripheral vessels and prevents them from responding to transfusion. Weakness of the antibacterial defense can presumably result from tissue hypoxia alone. But the presence of a leucotoxin in the blood must add to the damage to the antibacterial defense. Thus the way is open for the production of endotoxins from invading bacteria, whether the leucotoxin itself is or is not an endotoxin. Consequently there is reason to assume that a vicious circle is set up, and that toxin begets more toxin, with resulting injury to the peripheral vessels which is indistinguishable from that known to be caused by the direct injection of endotoxins into a healthy animal(5).

According to this thesis the toxicity of the plasma of the shocked rabbit just before death should be greater than that of the plasma of the rabbit which is still reversible to transfusion. We have already referred to evidence that this is so in the dog, but we do not find it so in the rabbit. This may be because our assay technic is too crude, or because there is in fact no necessary relationship between the amount of circulating toxin and the development of irreversibility to transfusion in the rabbit. For in this species we have found that the effect of a given amount of endotoxin on the peripheral vascular system is related not only to the dose of endotoxin, but to the sensitivity to the endotoxin, which increases enormously as the shock continues (7).

Summary and conclusions. 1) Normal macrophages from the peritoneal cavity of the normal and shocked rabbit, and granulocytes from the irritated peritoneal cavity of the normal rabbit show no significant morphologic injury after immersion in normal rabbit plasma for one hour at 37°C. But after immersion in plasma from rabbits in hemorrhagic shock, a considerable percent of the

cells show morphologic injury. A large number of granulocytes show changes similar to those characteristic of the L.E. cells. These changes are reversible if the shock plasma is replaced by normal plasma. 2) Shock plasma produces a severe depression of the phagocytic index; the percent of bacteria ingested is some 40% less than in normal plasma, and the percent of cells containing bacteria is some 30% less than in normal plasma. Shock plasma also reduces the bacteriostatic power of the phagocytes. The granulocyte in this respect appears to be damaged more severely than the macrophage. The extent of the injury produced by the plasma from rabbits in advanced shock appears to be no greater than that produced by the plasma of rabbits after two hours of shock, *i.e.* while they are still responsive to transfusion. The injurious property of plasma of the reversibly shocked rabbit persists, with undiminished potency, for at least four hours after transfusion. 3) It is concluded that a leucotoxin develops in the blood of the rabbit in hemorrhagic shock, and that this leucotoxin severely impairs the antibacterial potential of the animal.

1. Schweinburg, F. B., Yashar, J., Aprahamian, A., Davidoff, D., and Fine, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 587.

2. Rutenburg, S. H., and Fine, J., *ibid.*, 1956, v91, 217.

3. Smith, M. R., and Wood, W. B., Jr., *J. Exp. Med.*, 1947, v86, 257.

4. Fine, J., *The Bacterial Factor in Traumatic Shock*, pp43-46, C. C. Thomas, Springfield, Ohio, 1954.

5. Thomas, Lewis, *Physiological Disturbances Produced by Endotoxin. Ann. Rev. Physiol.*, 1954, v16, 467.

6. Fine, J., *N. Y. Acad. Sci.*, in press.

7. Schweinburg, F. B., and Fine. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 589.

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Reduction of Androstane-3,17-dione by Liver Homogenates.* (22795)

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Androstane-3, 17-dione is formed *in vitro* from androstane-3α, 17β-diol by homogenates of the liver and kidney of the rat, guinea pig and rabbit(1), from 4-androstene-3, 17-dione by guinea pig liver and kidney homogenates (2) and from androsterone and epiandrosterone by guinea pig liver homogenates(3). Furthermore, androstane-3, 17-dione has been postulated as the precursor of urinary androsterone and epiandrosterone(4). It would appear that androstane-3, 17-dione occupies a key position in the intermediary metabolism of androgens. Therefore, the metabolism of this substance by liver homogenates has been investigated.

Methods and procedures. Purification of androstane-3, 17-dione.† The material was submitted to adsorption chromatography on alumina (washed Harshaw). Those fractions which showed only androstane-3, 17-dione on paper chromatography (0.5 mg/spot) were pooled, treated with Darco charcoal, and recrystallized from acetone and water until a constant melting point was obtained. The purity of the material used in most of the experiments was tested further by infra-red analysis.‡ *DPNH and FDP.*§ The DPNH

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† The steroids were generously provided by Ciba Pharmaceutical Products, Inc., and Syntex, S. A.

‡ Analyses were performed with a Perkin-Elmer, Model 21, Infra-red Spectrophotometer.

§ The following abbreviations are used: DPN, diphosphopyridinenucleotide (Pabst Laboratories); DPNH, reduced DPN; FDP, fructose diphosphate (Nutritional Biochemicals Company); NA, nicotinamide (General Biochemicals, Inc.).