THE GRANULOCYTOPENIC RESPONSE IN HEMORRHAGIC SHOCK *

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When endotoxins are given intravenously the granulocytes leave the active stream of the circulation and congregate chiefly in the capillaries of the lung, spleen and liver. The same phenomenon can be expected to occur in hemorrhagic shock because experimental evidence suggests that the loss of the antibacterial defenses in shock results in the development of an endotoxemia (1). This paper presents the results of a comparative study of the circulating granulocytes in hemorrhagic shock and in endotoxemia, including data concerning the possible mechanisms involved.

METHOD

White adult albino rabbits and mongrel dogs were employed in this study. Endotoxemia was induced by injecting an *Escherichia coli* endotoxin (Difco Batch No. 0127-B8) intravenously. In rabbits this endotoxin was found to have an MLD per 100 of 2 mg. per Kg. and an MSD per 100 (MSD = maximal surviving dose, *i.e.*, the largest dose resulting in a zero mortality) of 0.05 mg. per Kg. These toxicity values were verified in rabbits prior to each experiment. Dogs were tolerant to at least 10 rabbit MLD's per 100 of this endotoxin. Hemorrhagic shock was induced as described elsewhere (2).

Total and differential counts of the blood were made in the normal state, and at different intervals after inducing shock or endotoxemia, beginning as early as one minute after challenge, again after 15, 30 or 60 minutes, and again every hour for several hours. Occasionally in survivors the count was obtained 24 hours later. The counts were made on blood from a large vein or artery according to convenience. There was no significant difference in the counts of venous and arterial blood.

Leukocyte counts were made in a variety of circumstances as follows:

I. Effect of variation of dose of endotoxin in normal rabbits. The response to four different doses of endotoxin (0.0002, 0.0004, 0.8 and 1.5 of one MLD per 100) was observed in four groups of four or more rabbits each. The purpose of this range of dosage was to observe the severity and duration of the granulocytopenia in response to a very light and a very severe injury.

II. Effect of tolerance to endotoxin. Tolerance was induced in normal rabbits by a daily intravenous injection of 0.07 MLD per 100 for seven successive days. Tolerance signifies an enhanced capacity to detoxify endotoxin. If the flight of the granulocytes is a direct effect of unneutralized endotoxin one might expect the granulocyte count to drop less severely or not at all in the tolerant animal; and if a drop occurs, that return to a normal count should occur more rapidly than in the nontolerant animal. Counts were also made each day before and after injection of the endotoxin in order to observe any change in response as tolerance develops.

III. Effect of hemorrhagic shock. Transfusion for shock of one and one-half hours' duration usually results in recovery (reversible shock). Transfusion for shock of four to six hours' duration is usually futile and death follows (irreversible shock). If the thesis is correct that irreversibility is due to the accumulation of a lethal dose of toxin, the curve of the granulocyte count for reversible and irreversible shock might resemble the respective curves for a sublethal and a lethal dose of toxin. Accordingly, hemorrhagic shock was produced and counts were taken before inducing shock, and at intervals before and after transfusion for shock of one and one-half hours' duration, and for shock of six hours' duration.

IV. Effect of plasma from irreversibly shocked rabbits in normal recipients. If shock plasma contains endotoxin, the granulocyte response in a normal recipient of such plasma (10 to 12 ml.) should resemble that in response to injected endotoxin. The extent and duration of the granulocytopenia might be a rough measure of the amount and toxicity of the endotoxin in the shock plasma.

The plasma was obtained as follows: Rabbits in hemorrhagic shock produced by the technique described (2) were transfused with all of their own shed blood after six hours, and when the resulting pressor response failed the rabbit was exsanguinated. The heparanized blood collected in sterile containers was promptly centrifuged, the plasma tested at once, or stored immediately thereafter in sterile containers at 4° C., and tested later. (Storage at this temperature for days or weeks does not reduce the initial toxicity of such plasma.)

V. Effect of hemorrhagic shock pretreated with nonabsorbable antibiotics. As already reported, such pre-

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treatment prevents the plasma of the animal in shock from becoming toxic to a vulnerable recipient (1). The granulocyte response to such plasma should differ from the response to plasma from shocked animals not given antibiotics because of the presumed absence of endotoxin, and should reveal any effect of the shock process on the granulocyte count which is not due to endotoxin.

The antibiotics used were Bacitracin® (100,000 units) and polymyxin (100 mg.) given to rabbits in water by gavage daily for five successive days. At this time stool cultures were free of all flora except small numbers of *B. Proteus* and *Pseudomonas*. Several hours after the fifth dose hemorrhagic shock was induced and continued for six hours, after which the animal was transfused with its own shed blood.

VI. Effect of epinephrine. Immediate and severe vasoconstriction is produced by hemorrhagic shock or by endotoxin. Since there is reason to believe that endotoxin in the normal and shocked animal exerts its adverse effects on the peripheral vessels via epinephrine (3), the role of epinephrine in the granulocyte response was studied in two ways: *1*) by observing the effect of an intravenous injection of epinephrine (in normal rabbits 0.1 ml. of 1:100,000 dilution and in normal dogs, 0.8 ml. of 1:1,000 dilution), or of norepinephrine (1 ml. of 0.2 per cent solution); and *2*) by the use of adrenergic blockade. The latter approach is more appropriate to the problem because, if epinephrine plays a role in the granulocyte response in hemorrhagic shock or endotoxemia, it is presumably acting more or less continuously. But because a satisfactory method for continuous and prolonged administration of epinephrine that the rabbit will tolerate is not yet available, the role of this substance acting continuously should be disclosed inferentially by the response to dibenamine. Dibenamine was given to rabbits (20 mg. per Kg.) two to three hours prior to a dose of endotoxin (1.2 MLD per 100 or a 0.0004 MLD per 1000), and prior to hemorrhagic shock terminated by transfusion after one and one-half hours.

RESULTS (TABLES I THROUGH VI)[1]

The total count and the granulocyte count in any single observation are given as the mean values for the group. In no case were there less than four animals in a group. Changes in response to treatment are expressed in per cent of the pretreatment level.

Experiment 1—Effect of variation in dose of endotoxin

Table I demonstrates that in rabbits the granulocyte count falls to an extremely low level. This occurs almost immediately (one minute). After a lethal dose of endotoxin significant recovery from the fall does not occur at any time before death. The extent of the fall is less the smaller the dose, and the speed of recovery tends to be in

[1] The "approximate standard error" and "per cent of the pretreatment level" for each observation in the tables was calculated by Miss Rita Nickerson under the supervision of Dr. Mindel C. Sheps of the Department of Preventive Medicine of the Harvard Medical School. The procedure employed is described by her as follows:

Analysis of variance on the logarithms of the counts was performed on the data from each experiment separately. The sequential mean values shown in the table for each particular experiment are, therefore, the geometric means of the counts observed.

The standard errors of the mean log counts were calculated from the residual error term in each analysis of variance. The transformation into antilogarithms may be illustrated on the following values from the granulocyte counts observed in Experiment III, A on rabbits.

	Mean	S. E.	Mean ± 1 S. E.
At onset:			
Logs	3.726	0.108	3.618–3.834
Antilogs	5,321	1.284	4,150–6,830
At 1 hour:			
Logs	2.632	0.108	2.524–2.740
Antilogs	429	1.284	334–550

Since the calculations were done on the log counts the standard error of a mean can be stated precisely only in terms of logs. For presentation in terms of cell counts, "approximate standard errors" were computed in two ways:

a) From the antilogarithm of the standard error (1.284) as shown above, the per cent standard error is approximately 100(1.284 − 1) = 28 per cent (see Table III, A) (4).

b) In terms of cell counts themselves the "approximate standard error" of any mean in this experiment is 28 per cent of this mean. Therefore, at the onset the approximate standard error is (0.28) (5,321) = 1,490 cells and at one hour it is (0.28) (429) = 120 cells.

Each experiment consisted of repeated observations made on each of several animals. The experimental results, therefore, were considered not by comparing absolute counts, but by noting the logarithm of the per cent change as shown in the tables. To test the difference between the values at one hour, say, in two separate experiments, therefore, the following procedure was adopted:

	Mean log count at onset minus mean log count at 1 hour	(Standard error of difference)[2]
Rabbits' granulocytes Table III, A	1.094	0.0234
Rabbits' granulocytes Table III, C	0.448	0.0072
Difference in one hour values	0.646	0.0306

$$t = \frac{0.646}{\sqrt{0.0306}} = 3.69 \qquad p < 0.01$$

TABLE I

Effect of various doses of an E. coli endotoxin on total number of leukocytes and of circulating granulocytes in the normal adult rabbit (seven to eight animals in each group)

Endotoxin		Mean no. of granulocytes/ cu. mm.	Approximate S. E. (13%)	Per cent of pretreatment level	Mean of total white cells/ cu. mm.	Approximate S. E. (8%)	Per cent of pretreatment level
Dose of	Time						
	hrs.						
0.0002 MLD	0	5,309	690		8,830	706	
	1	1,523	198	29	4,943	395	56
	2	4,416	574	83	7,430	594	84
			(17%)			(8%)	
0.014 MLD	0	5,781	983		8,511	681	
	1	582	99	11	2,897	232	34
	2	649	110	11	2,577	206	30
	3	1,774	302	31	4,150	332	48
			(34%)			(14%)	
0.8 MLD	0	4,227	1,437		6,903	966	
	2	309	105	7.3	2,075	290	30
	4	505	172	12	2,697	378	39
			(38%)			(15%)	
1.5 MLD	0	2,685	1,020		6,040	906	
	¼	275	104	10	2,710	406	45
	1	121	46	4.5	2,109	316	44
	2	129	49	4.8	1,548	232	26
	3	140	53	5.2	1,664	250	28
	4	268	102	10	1,714	257	28

rough inverse proportion to the dose. In survivors an overshoot occurs in all instances within 24 hours, and often within six to eight hours after small doses.

The fall in the total count is due largely to the fall in the granulocytes. With all doses there is a transient absolute lymphocytosis, which disappears within an hour or two. Following large doses there may be a lymphocytopenia by the third or fourth hour.

In concurrent experiments on the rabbit, to be reported, the granulocytes leaving the circulation, as already noted by others (5, 6), were seen congregating chiefly in the capillaries of the lung,

TABLE II

Leukopenic response to 0.07 MLD E. coli endotoxin in nontolerant and tolerant rabbits (five animals in each group)

Time	Mean no. of granulocytes	Approximate S. E. (17%)	Per cent of pretreatment level	Mean of total white cells	Approximate S. E. (12%)	Per cent of pretreatment level
hrs.			Nontolerant			
0	5,420	921		8,129	975	48
½	1,242	211	23	3,945	473	21
1	389	66	7.2	1,698	204	32
2	361	61	6.7	1,812	260	22
3	468	80	8.6	1,762	211	22
4	908	154	17	1,941	233	24
5	1,689	287	37	3,296	396	40
6	3,837	652	71	6,886	826	85
			Tolerant			
		(14%)			(8%)	
0	9,730	1,362		13,303	1,064	
½	698	98	7.2	3,750	300	28
1	2,831	396	29	5,807	465	44
2	6,339	887	65	8,790	703	66

TABLE III

Leukopenic response to reversible and irreversible hemorrhagic shock in the dog and rabbit *

Time	Mean no. of granulocytes	Approx. S. E. 28% (23%)	Readings as % of pretreatment level	Mean total white cell count	Approx. S. E. 15% (17%)	Readings as % of pretreatment level
			A. Reversible shock†			
hrs.						
0	5,321 (7,585)	1,490 (1,744)		8,110 (10,422)	1,216 (1,771)	
1/6	1,247 (5,012)	349 (1,153)	23 (66)	4,842 (8,750)	726 (1,488)	60 (84)
1/2	457 (3,483)	128 (801)	8.6 (46)	3,589 (6,607)	538 (1,123)	44 (63)
1	429 (3,990)	120 (918)	8.1 (53)	3,614 (7,620)	542 (1,295)	45 (73)
4	2,162 (6,998)	605 (1,610)	41 (92)	6,576 (12,302)	986 (2,091)	81 (118)
			B. Irreversible shock‡			
		39% (18%)			13% (12%)	
0	4,169 (2,790)	1,626 (2,302)		7,551 (17,660)	982 (2,119)	
1/6	767 (5,544)	299 (998)	13 (43)	5,035 (9,955)	655 (1,195)	67 (56)
1/2	396 (4,074)	154 (733)	9.5 (32)	3,565 (8,200)	463 (991)	47 (47)
1	281 (5,755)	110 (1,036)	6.7 (45)	3,963 (10,715)	515 (1,286)	42 (61)
2	658 (5,285)	257 (951)	16 (41)	4,488 (9,185)	583 (1,102)	59 (52)
6	813 (6,290)	317 (1,246)	20 (54)	4,677 (18,115)	608 (2,174)	60 (103)
			C. 6 hour shock in endotoxin-tolerant rabbits (5 expts.)			
		15%			11%	
0	6,487	973		9,638	1,060	
1/2	2,260	339	35	4,634	510	48
1	2,312	347	36	5,035	554	52
2	3,614	542	56	6,486	713	67
3	5,152	773	79	8,280	911	86
6	5,597	840	86	8,830	971	92

* Figures in parentheses apply to dogs; figures not in parentheses apply to rabbits.
† Four dogs, eight rabbits.
‡ Six dogs, four rabbits.

liver and spleen. With the return of the total and granulocyte count toward normal, many of the circulating granulocytes in stained smears were young forms, and there was a noteworthy concurrent rise in the number of normoblasts. Hence new cells from the bone marrow replaced the segregated cells, many of which were still *in situ* 24 hours later, undergoing lysis, or ingestion by macrophages.

Dogs withstood 10 rabbit MLD's per 100 of the Difco endotoxin without noticeable effect, and without a fall in the total or granulocyte count. Larger doses were not tried.

Comment. Thus it appears that in rabbits, many, if not all, the granulocytes which leave the axial stream are rendered functionless permanently, presumably as a result of injury by the injected endotoxin. Failure of recovery of a normal count when the dose of endotoxin is lethal may mean suppression of bone marrow function by the endotoxin, or damage by circulating endotoxin to young granulocytes, and resultant segre-

TABLE IV

Leukopenic response of normal rabbits to plasma (10 to 12 ml.) from irreversibly shocked rabbits * (*five experiments*)

Time	Mean total of granulocytes	Approx. S. E. 36%	Readings as % of pretreatment level	Mean total of white cells	Approx. S. E. 12%	Readings as % of pretreatment level
hrs.						
0	5,224	1,881		8,204	984	
1	1,549	558	30	3,873	465	47
2	2,767	996	50	5,495	659	67
3	3,435	1,237	66	5,890	707	22

* Leukopenic response of normal rabbits to normal plasma is insignificant.

gation as rapidly as they are released into the circulation.

Experiment 2—Effect of tolerance to endotoxin

The fall in the total and granulocyte count immediately after administration of the endotoxin was about the same with each successive daily injection for seven days. But, as Table II shows, while the initial leukopenic response is the same in tolerant and nontolerant rabbits, the rate of return to normal increases with the acquisition of tolerance. After two hours the granulocyte count is some 90 per cent below the control value in the nontolerant rabbit, but it is only 35 per cent below in the tolerant rabbit.

Comment. The rate of delivery of granulocytes to the circulation from the bone marrow and the segregation of circulating granulocytes in the lungs, liver and spleen appears to be dependent on the speed of detoxification of the endotoxin.

Experiment 3—Leukopenic response to hemorrhagic shock

A. In rabbits. The granulocytopenic response to irreversible hemorrhagic shock (Table III, B) in rabbits is quite comparable to the response to a lethal dose of endotoxin, except that the absolute lymphocytosis, unlike the granulocytopenia, does not persist until death. The extreme depletion of granulocytes is evident within one minute after inducing shock. In reversible shock return to the normal count begins shortly after transfusion (Table III, A). By the next day there is a leukocytosis, and the granulocyte count is far above normal. The shed blood in the reservoir which is used for the transfusion shows a moderate drop in the total and granulocyte count before it is transfused. This drop is probably accounted for by the fact that the granulocytopenia is present before most of this blood has left the circulation. Even if the transfused leukocytes, which are functionally intact by *in vitro* test (7), remain in the axial stream, they do not increase the count.

TABLE V

Granulocytopenic response during hemorrhagic shock in rabbits pretreated with antibiotics (six experiments)

Time	Mean no. of granulocytes	Approximate S. E. 23%	Readings as % of pretreatment levels
hrs.			
0	4,820	1,109	
1/12	212	49	4.4
1/2	158	36	3.3
1	240	55	5.0
2	1,390	320	28.8
3	2,930	624	60.8
4	3,240	745	67.2

Comment. Rabbits tolerant to endotoxin are also tolerant to hemorrhagic shock, and when exposed to hemorrhagic shock their plasma does not develop a factor toxic to a vulnerable recipient (7). If endotoxins are the chief cause of granulocytopenia in hemorrhagic shock, one would expect the granulocytes in tolerant rabbits in shock to behave as they do when given endotoxin (*cf.* Experiment 2). Table III, C shows that this expectation was confirmed, *i.e.*, the granulocytopenia is relatively less severe in animals tolerant to hemorrhagic shock than in those not tolerant. By the end of the second hour return toward normal is already in evidence, and is near normal when shock is terminated by transfusion at six hours (7).

TABLE VI

Leukopenic response of dogs and rabbits to single I.V. dose of epinephrine *

Time	Mean granulocyte count	Approx. S. E. 29% (18%)	Readings as % of pretreatment level	Mean total white cell count	Approx. S. E. 12% (13%)	Readings as % of pretreatment level
hrs.						
0	4,677 (12,676)	1,356 (2,282)		7,925 (17,220)	951 (2,239)	
1/6	2,952 (10,900)	856 (1,962)	63 (80)	8,184 (14,723)	982 (1,914)	103 (86)
1/2	1,932 (6,545)	560 (1,178)	41 (52)	6,855 (10,422)	823 (1,355)	86 (61)
1	1,449 (5,755)	420 (1,036)	31 (36)	6,775 (9,954)	813 (1,294)	86 (58)
2	1,991 (7,535)	577 (1,356)	43 (59)	5,901 (11,272)	708 (1,465)	74 (65)
4	4,865 (13,020)	1,411 (2,344)	103 (103)	7,889 (17,200)	947 (2,236)	99 (99)

* Figures in parentheses are for nine dogs; figures not in parentheses are for seven rabbits.

B. In dogs. The general trend is the same in dogs as in rabbits, but it is far less marked in the dog (Tables III, A and B).

Comment. If leukopenia is caused by endotoxin in the dog as well as in the rabbit, the lesser response in the dog may reflect a more efficient bone marrow response, a less vulnerable granulocyte, or a lesser amount of circulating endotoxin. The latter seems unlikely if the gross pathology of the shock (especially the hemorrhagic necrosis of the intestinal mucosa) is an index of the severity of the endotoxemia.

Experiment 4—Effect of toxic plasma from irreversibly shocked rabbits injected into normal recipients

Normal plasma did not produce a fall in the total or granulocyte count.

The response to a single dose of toxic plasma is like that to a small dose of endotoxin (Table IV). In no instance did the low granulocyte count last longer than four hours.

Daily doses of toxic plasma given for seven successive days produced the same initial fall in the granulocyte count, but by the fifth day, as with repeated daily doses of endotoxin, the count returned to normal more rapidly than in non-tolerant rabbits. The more rapid return to normal in rabbits tolerant to toxic plasma suggests a more rapid removal of endotoxin from injected plasma.

Experiment 5—Effect of hemorrhagic shock pretreated with nonabsorbable antibiotics

The initial fall in the total and granulocyte count is no less severe in these than in untreated rabbits (*cf.* Table III), but the counts begin a return to normal in two hours. Since the initial fall is in excess of what would appear to be caused by epinephrine alone (see below), one suspects that the blood is not free of endotoxin in these protected animals. From evidence to be published elsewhere there is reason to believe that shortly after giving antibiotics there is a rapid rise in the amount of endotoxin entering the circulation, and, therefore, that the protective effect of antibiotics given daily for several days may be owing to induced tolerance rather than to the prevention of endotoxemia. If this is the case, the severe granulocytopenia in these experiments may be analogous to the granulocyte response in endotoxin-tolerant rabbits as shown in Table II.

Experiment 6—Effect of epinephrine and of norepinephrine

A. In the rabbit (Table VI). The dose employed (0.1 ml. of 1:10,000), or a larger dose, produces a granulocytopenia that is less severe and more transient than that resulting from a very small dose of endotoxin. Thus one may conclude that the granulocytopenic curve in hemorrhagic shock reflects the action of epinephrine as well as endotoxin.

B. In the dog. In the dog the dose employed (0.8 ml. 1:1000) produced no change until after the transient period of hypertension, when the response was quite like that in the rabbit; but recovery was a little more rapid than in the rabbit (Table VI).

Norepinephrine had no effect on the granulocyte response.

C. Effect of pretreatment with dibenamine (rabbit). This drug given in a dose of 20 mg. per Kg. produces maximal adrenergic blockade within 25 minutes. In the normal animal it produces no immediate effect on the count, but a leukocytosis appears after 24 hours.

Epinephrine given three hours after dibenamine failed to evoke a granulocytopenia. But endotoxin in both sublethal (0.0004 MLD per 100) and lethal (1.2 MLD per 100) doses produced the same changes as in the non-dibenaminized rabbit. Whereas two of five rabbits receiving the lethal dose survived, there were no survivors among the non-dibenaminized rabbits receiving this dose. The two survivors showed a marked leukocytosis after 24 hours.

In hemorrhagic shock dibenamine did not prevent a profound and persistent granulocytopenia in four rabbits, but three showed no granulocytopenia and two showed a very transient granulocytopenia (30 minutes).

DISCUSSION

The flight of granulocytes from the circulating blood of the rabbit in response to the intravenous injection of endotoxin has been previously described by others, notably Delauney and associates (5) and Bennett and Beeson (8). Delauney

stated that the onset of the leukopenia occurred one hour after intravenous injection. Berthrong and Cluff (9) observed that the granulocytopenia following sublethal doses of endotoxin was maximal within one to two hours, and was replaced by a polymorphonuclear leukocytosis at six to seven hours. Following sublethal or lethal doses of endotoxin we have observed that the granulocytopenia is maximal or nearly so within a few minutes.

The striking parallelism between the changes in the total and differential counts in experimental endotoxemia and in hemorrhagic shock, both in time of onset and degree and duration, suggests a common mechanism in both. The immediate granulocytopenic response to endotoxin signifies a direct effect of endotoxin on the granulocytes, or on the vessels to which they adhere. Since this occurs even in the dibenaminized animal it cannot be, as Delauney (5) believes, a wholly epinephrine-dependent phenomenon.

If this is so, and the phenomena involved are the same in hemorrhagic shock, one must conclude that an endotoxemia is present within minutes after induction of hemorrhagic shock. That this is the case is also suggested by recent experiments to be reported showing that in thorotrast-pretreated rabbits hemorrhagic shock of only *five minutes' duration* elicits a severe Shwartzman reaction and causes death. If the immediate and severe granulocytopenic response in hemorrhagic shock, like the granulocytopenia of endotoxemia, cannot be explained as being wholly or primarily due to epinephrine, the prevention of the granulocytopenic response in five of nine dibenaminized animals in hemorrhagic shock would seem to be owing to some property of dibenamine not related to adrenergic blockade.

One cannot exclude the possibility that a leukopenic agent other than endotoxin or epinephrine may be operating in hemorrhagic shock. The fact that the leukopenia occurs so rapidly after the onset of shock would appear to exclude any leukopenic agent which develops only in consequence of tissue injury caused by prolonged duration of the shock state. Until evidence for some other type of leukopenic agent is forthcoming, it appears that the flight of granulocytes in hemorrhagic shock can be considered to be mainly a manifestation of endotoxemia. According to Braude and co-workers (10, 11) endotoxin attaches to the granulocytes, which then move to the liver and lungs, and so acts to transport endotoxin to the R.E. system. We have observed the deposition of the leukocytes in the capillaries of the liver, lung and spleen almost immediately after inducing shock or injecting toxin. Many of them can be seen *in situ* 24 hours later in process of disintegration or phagocytosis by macrophages. These cells are functionally damaged because they do not move to a site of challenge (*e.g.*, to bacteria injected into the lung or liver just prior to the flight of the granulocytes[2]). From *in vitro* observations we know that endotoxin or shock plasma produces a severe though reversible morphologic injury, which may make the cell more readily available for ingestion by the R.E. cells; but in addition, the granulocytes exhibit a severe depression of their phagocytic and bacteriostatic capacity (6). These observations indicate that the flight of these cells is not a protective phenomenon, unless perchance the flight is an essential means for delivery of endotoxins to the R.E. system. The deposition of the granulocytes in the R.E. system, though probably achieved by an increase in the "stickiness" of the capillary walls, resembles the process of agglutination described by Wood and Smith in the response of circulating granulocytes to a local inflammatory stimulus (12). But in the latter case the adherent cell manages to penetrate the capillary wall, reach the site of challenge, and continue to function. Meanwhile there is no dearth of circulating cells to serve the defense needs of other areas. In shock, on the other hand, all other areas are less well defended by virtue of the low circulating count, and the inability of the bone marrow to supply the deficit. In human shock leukopenia or leukocytosis has been observed (13). Presumably this depends on the state of bone marrow activity preceding the onset of the shock state, and the sensitivity of this system to depression by circulating toxins. In the rabbit this sensitivity is high, in the dog it is less, and in man the observations are too few to provide a definite picture.

SUMMARY AND CONCLUSIONS

The circulating granulocytes respond to the intravenous injection of endotoxin or to hemor-

[2] Unpublished observations.

rhagic shock by an almost immediate flight into the capillaries of the lung, liver and spleen, where they appear to remain and eventually undergo autolysis or phagocytosis by macrophages. The epinephrine response to shock or to endotoxin also produces a flight of the granulocytes. But because the flight due to epinephrine alone is much less severe and more transient, it cannot wholly account for the phenomenon. This is further demonstrated by the fact that dibenamine does not block the flight induced by endotoxins. But because dibenamine frequently minimizes or prevents the extreme granulocytopenic response to hemorrhagic shock, its protective effect may lie not only in its anti-epinephrine effect, but also in the preservation of the detoxifying potential of the tissues by virtue of the better blood flow which this drug effects in hemorrhagic shock. The altered granulocytopenic response to endotoxin in animals tolerant to endotoxin and to hemorrhagic shock is attributable to the very rapid detoxification of endotoxin in such animals.

REFERENCES

1. Schweinburg, F. B., Shapiro, P. B., Frank, E. D., and Fine, J. Host resistance to hemorrhagic shock. IX. Demonstration of circulating lethal toxin in hemorrhagic shock. Proc. Soc. exp. Biol. (N. Y.) 1957, **95**, 646.
2. Fine, J., Frank, H. A., Schweinburg, F. B., Jacob, S., and Gordon, E. The bacterial factor in traumatic shock. Bull. N. Y. Acad. Sci. 1952, **55**, 429.
3. Thomas, L. Role of epinephrine in reactions produced by endotoxins of gram-negative bacteria. I. Hemorrhagic necrosis produced by epinephrine in the skin of endotoxin-treated rabbits. J. exp. Med. 1956, **104**, 865.
4. Bliss, C. I. The Statistics of Bioassay. New York, Academic Press Inc., 1952, p. 506.
5. Delauney, A., Boquet, P., Lebrun, J., Lehoult, Y., and Delauney, M. La Mode d'Action des Endotoxins bactériennes. IV. Les troubles vaso-moteurs chez les animaux intoxiqúes et leurs conséquences. J. Physiol. (Paris) 1948, **40**, 89.
6. Rutenberg, S. H., and Fine, J. Resistance to bacteria in hemorrhagic shock. VII. Demonstration of leukotoxin in plasma of the shocked rabbit. Proc. Soc. exp. Biol. (N. Y.) 1956, **93**, 484.
7. Smiddy, F. G., and Fine, J. Host resistance to hemorrhagic shock. X. Induction of resistance by shock plasma and by endotoxins. Proc. Soc. exp. Biol. (N. Y.) 1957, **96**, 558.
8. Bennett, I. L., Jr., and Beeson, P. B. Properties and biological effects of bacterial pyrogens. Medicine 1950, **29**, 365.
9. Berthrong, M., and Cluff, L. E. Studies of the effect of bacterial endotoxins on rabbit leukocytes. I. J. exp. Med. 1953, **98**, 331.
10. Braude, A. I., Carey, F. J., Sutherland, D., and Zalesky, M. Studies with radioactive endotoxin. I. The use of Cr^{51} to label endotoxin of *Escherichia coli*. J. clin. Invest. 1955, **34**, 850.
11. Braude, A. I., Carey, F. J., and Zalesky, M. Studies with radioactive endotoxin. II. Correlation of physiologic effects with distribution of radioactivity in rabbits injected with lethal doses of *E. coli* endotoxin labelled with radioactive sodium chromate. J. clin. Invest. 1955, **34**, 858.
12. Wood, W. B., Jr., and Smith, M. R. Surface phagocytosis—Its relation to the mechanism of recovery in acute pneumonia caused by encapsulated bacteria. Trans. Ass. Amer. Physcns 1947, **60**, 77.
13. Davis, H. A. Shock and Allied Forms of Failure of the Circulation. New York, Grune and Stratton, 1949, p. 92.