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William B. Herring, … , Richard I. Walker, Jeffress G. Palmer

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Research Article

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DISTRIBUTION AND CLEARANCE OF CIRCULATING ENDOTOXIN *

By WILLIAM B. HERRING, JOHN C. HERION, RICHARD I. WALKER,† AND JEFFRESS G. PALMER

(From the Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, N. C.)

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Bacterial endotoxins injected intravenously induce characteristic changes in body temperature and numbers of circulating leukocytes. A decrease in the duration and intensity of these effects occurs after repeated injection of similar doses (1, 2). This state of relative refractoriness to endotoxin, called "tolerance," is nonspecific for a single endotoxin and is unrelated to the presence of antibodies (3–5). Tolerant animals given thorotrast or other colloids exhibit a nontolerant febrile response to endotoxin. This has led to the hypothesis that tolerance results from more rapid clearance of endotoxin by the reticuloendothelial system (4).

Understanding the mechanisms of endotoxin action requires knowledge of the distribution and fate of injected endotoxin. Chromium^{51}-labeled endotoxin was shown to localize rapidly in liver and the buffy coat of blood; smaller amounts were found in the lungs, spleen, and other organs (6). Blood clearance of large amounts of Cr^{51} endotoxin was shown to be more rapid in tolerant than in nontolerant rabbits. Although clearance of small doses was slightly more rapid in tolerant animals, this was not considered significant (7). Endotoxin was given, however, in doses 10 to 600 times greater than those commonly used to study fever and leukocyte changes.

The availability of chromium^{51} of high specific activity has made feasible the study of clearance of small doses of Cr^{51} endotoxin and its distribution among blood elements. This paper reports the results of such studies in nontolerant and tolerant animals.

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MATERIALS AND METHODS

New Zealand white rabbits weighing 1.9 to 3.5 kg were studied. To produce febrile tolerance, unlabeled endotoxin, 3 to 4 μg per kg, was given intravenously to one group of animals for five consecutive days before administration of labeled endotoxin (2). Temperature measurements on four rabbits demonstrated definite febrile tolerance after the third injection; therefore it was considered unnecessary to test each animal for tolerance.

The endotoxin (*E. coli* lipopolysaccharide, lot 026: B6, Difco Laboratories, Detroit) was labeled *in vitro* by an adaptation of the method of Braude, Carey, Sutherland, and Zalesky (8). Endotoxin, 69 to 207 μg per ml of sterile phosphate buffer (pH 7.0), was incubated with $Cr^{51}Cl_3$ (Chromitope, Squibb) at 37° C for 48 hours. The concentration of chromium ranged from 0.24 to 1.25 μg per ml. After incubation the mixture was dialyzed at room temperature against phosphate buffer (pH 7.0) for 96 to 112 hours. After dialysis the labeled endotoxin was diluted with phosphate buffer to a final concentration of 10 μg per ml (Table I).

Labeled endotoxin, 3 to 4 μg per kg of body weight, was injected into a marginal ear vein. Each animal was lightly anesthetized with intravenous pentobarbital sodium 1 to 2 minutes before bleeding. Blood samples, collected by cardiac puncture, were placed into siliconized test tubes containing 0.1 ml 10 per cent disodium EDTA (sequestrene) per 10 ml of blood. Samples were obtained at 10 seconds, 30 seconds, 1, 2, 3, 5, and 8 minutes after injection of Cr^{51} endotoxin; in some animals samples were drawn at greater intervals. The 10- and 30-second samples, 8 to 10 ml each, were obtained from the same animal without removing the intracardiac needle. The 1- and 2-minute and some 3- and 5-minute samples were collected in a similar manner. The remaining samples, 20 ml each, were obtained from individual rabbits.

On each blood sample, the volume of packed red cells (VPRC) was measured in a Wintrobe hematocrit tube, platelets were counted by phase microscopy, and total leukocytes were determined with a Coulter electronic cell counter. A differential count of 200 leukocytes was performed from coverslip smears stained with Wright's stain.

Plasma free of formed elements was obtained by centrifugation of blood at 800 *g* for 30 minutes at 5° C. In each instance this was completed within 1 hour after collection. Plasma samples separated from portions of

TABLE I

Labeling of endotoxin

Preparation no.*	Endotoxin added to Cr	Chromium			μg Cr bound/ μg endotoxin	Specific activity endotoxin
		Specific activity	Added	Bound		
	μg	μc/μg	μg	μg		cpm/μg
1	1,122	72.9	6.86	5.42	0.00483	18,618
2	1,683	49.8	10.18	8.35	0.00496	17,677
3	990	290.0	3.64	3.46	0.00349	36,176
4	1,080	150.0	3.67	2.86	0.00264	23,210

* A single lot of *E. coli* lipopolysaccharide (026:B6, Difco) was used for all preparations.

the same blood sample at intervals up to 2 hours after collection showed no significant gain or loss of radioactivity. After removal of plasma and buffy coat, red cells were washed four times in 2 volumes of isotonic saline and packed by centrifugation. The radioactivity of whole blood, plasma, and red cells was measured in a well scintillation counter; samples were counted long enough to insure a maximum counting error of 3 per cent. All counts were corrected for physical decay. Radioactivity was expressed as counts per minute per milliliter (cpm per ml) or as percentage of the total injected dose remaining in circulating blood or plasma. The latter was calculated from the blood volume (BV), which was assumed to be 5.8 per cent of body weight (9, 10), and from the plasma volume, derived by the formula $PV = BV\ [(100 - VPRC \text{ in per cent})/100]$.

The radioactivity of lymphocytes was determined directly on cells isolated from 70 to 90 ml of blood obtained from two nontolerant and two tolerant rabbits between 1 and 6 minutes after injection of Cr^{51} endotoxin. Separation of lymphocytes was performed by a modification of the method of Fichtelius (11, 12). Heparinized blood was incubated on cotton columns for one hour at 37° C. The cotton was then washed with a volume of warm isotonic saline equal to twice the volume of blood to elute red cells and lymphocytes. Red cells were removed by dextran sedimentation and gramicidin-lysolecithin hemolysis. The lymphocytes, virtually free of granulocytes and platelets, were washed and examined for radioactivity.

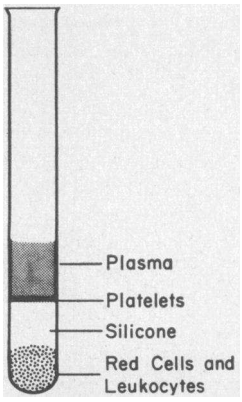

FIG. 1. SEPARATION OF BLOOD ELEMENTS BY SILICONE FLOTATION.

Two methods were used to determine radioactivity in leukocytes. First, blood samples, 30 ml each, were obtained from eight normal rabbits, four at 1 minute and four at 5 minutes after injection of 4 μg of Cr^{51} endotoxin per kg. The blood was mixed with ½ volume of 1 per cent EDTA in 0.5 per cent saline and centrifuged at 200 *g* for 25 minutes at 4° C. The supernates contained more than half the platelets, but less than 1 per cent of the leukocytes. The platelets were isolated from the supernates by centrifugation, washed twice with 1 per cent EDTA in 0.5 per cent saline after hemolysis of residual erythrocytes with 1 per cent ammonium oxalate, and their radioactivity was measured in a well counter. After removal of the platelet-rich supernates, the samples were added to 2 volumes of ice-cold 3 per cent dextran to facilitate red cell sedimentation. The leukocytes were then isolated from the dextran supernates by the method of Athens and associates (13).

Second, blood components were separated by an adaptation of the method of Morgan and Szafir (14). Two-ml volumes of a silicone mixture (Dow-Corning no. 555 and no. 200) with a specific gravity of 1.039 were placed in 15 × 125 mm test tubes. Four ml of blood were pipetted into the silicone and the tubes were centrifuged at 800 *g* for 20 minutes at 5° C. Sharp separation into four layers occurred (Figure 1). The top layer was cell-free plasma. Between the plasma and the clear silicone layer was a membrane consisting predominantly of platelets but containing some leukocytes, chiefly mononuclear cells. The few granulocytes present were mostly basophils. The plasma, platelet membrane, and silicone were removed, leaving the packed red cell layer which contained most of the leukocytes. The platelet membrane was fragile and occasionally adhered to the tube, preventing quantitative removal for measurement of radioactivity. The activity of this layer was therefore determined indirectly from reconstituted blood, prepared by adding to the red cell-leukocyte layer a volume of the animal's own cell-free plasma equal to that present in the original 4-ml blood sample. The volume of plasma to be added was calculated from the hematocrit determined on the same blood sample. The tube containing reconstituted blood was placed directly into the well counter to measure the ra-

dioactivity. Observations of mixtures of labeled plasma and unlabeled platelets, and labeled platelets and unlabeled plasma or saline showed that no significant transfer of radioactivity occurred *in vitro*.

Platelet counts and total and differential leukocyte counts were performed on the reconstituted blood to evaluate the procedure. Table II shows hematocrits and leukocyte and platelet counts on the original blood samples, and leukocyte and platelet counts on the reconstituted samples. With this method 97 per cent of the platelets were removed from the blood; the reconstituted samples retained 67 per cent of all leukocytes and 85 per cent of the granulocytes. The silicone layer contained no cells or radioactivity; the traces remaining in the blood samples did not interfere with cell counts.

RESULTS

Potency and stability of Cr^{51}-labeled endotoxin

After storage at room temperature for as long as 56 days, labeled endotoxin, 3 to 5 μg per kg, induced typical febrile and leukocytic responses in normal rabbits. Twenty ml of preparation 2 (Table I) was redialyzed after 33 days storage; only 0.33 per cent of the radioactivity was removed in 44 hours, indicating a stable bond between the Cr^{51} and endotoxin. These findings are consistent with those of Braude and associates (8).

Clearance of Cr^{51}-labeled endotoxin

The specific activity of whole blood and plasma after administration of Cr^{51} endotoxin is shown in Table III. Figure 2 shows the percentages of the injected radioactivity remaining in circulating blood and plasma at intervals up to 8 minutes after injection. In both nontolerant and tolerant animals, radioactivity disappeared rapidly from the circulation, 90 per cent being removed within

TABLE II

Alterations in concentration of formed elements produced by reconstitution of blood after centrifugation with silicone

Time after Cr^{51} endotoxin injection	Rabbit		VPRC *	Leuk./mm³		Gran./mm³		Plat./mm³ ×10⁻³	
	State	No.	%	Whole blood	Reconst. blood	Whole blood	Reconst. blood	Whole blood	Reconst. blood
min									
1	Nontol.	17	41.5	10500	4050	6195	2673	260	7.5
		18	33	6850	3500	2432	1645	292.5	15
		19	41	8100	4700	2187	1222	330	7.5
	Tol.	5	35	6700	5050	2077	2172	297.5	2.5
		6	35	5350	3200	1819	1376	272.5	7.5
3	Nontol.	21	35	9250	7200	2960	3132	177.5	20
		22	40	10400	6350	2600	2159	225	2.5
		23	31.5	5600	3500	2828	1558	295	5
		24	37	6800	3900	1836	1287	305	2.5
	Tol.	4	39	3500	1950	875	653	65	12.5
		7	36	7500	4750	1575	2161	195	2.5
		8	36.5	7200	3650	792	675	127.5	0
5	Nontol.	25	38	5850	4000	1960	1440	110	7.5
		27	32	6200	4400	2232	1980	222.5	5
		29	38	7550	5900	1737	1800	187.5	2.5
	Tol.	9	39	9600	5650	2016	1695	332.5	10
		10	30	8400	5600	3444	3444	365	5
		34	35	7300	6450	1716	1806	100	5
8	Nontol.	30	37	3750	3150	263	205	295	5
		31	34	5200	4050	676	446	382.5	7.5
		32	33.5	2900	2100	348	357	197.5	5
	Tol.	13	36.5	5800	4300	2668	2731	400	7.5
		14	35	5050	3950	1667	1975	170	2.5
		15	35	6100	4900	2654	3014	220	10
		16	34	5050	4900	758	980	187.5	2.5
Means of all values				6660	4446	2013	1703	240.5	6.4

* VPRC = volume of packed red cells.

TABLE III

Cr^{51} endotoxin clearance

Time after Cr^{51} endotoxin injection	Samples		Specific activity as cpm/ml ±SE			
			Whole blood		Cell-free plasma	
	Nontol.	Tol.	Nontol.	Tol.	Nontol.	Tol.
min	*no.*	*no.*				
$\frac{1}{6}$	5	4	2677 ±263	2296 ±110	3343 ±395	2052 ±202
$\frac{1}{2}$	9	9	2230 ±115	1818 ±158	2393 ±233	1353 ±132
1	8	11	1633 ±103	1098 ± 83	1680 ±175	771 ± 83
2	6	5	1001 ±131	393 ± 45	1061 ±179	309 ± 48
3	10	8	608 ± 58	192 + 28	659 ± 72	163 ± 28
5	8	9	263 ± 46	109 ± 27	300 ± 52	83 ± 13
8	8	10	135 ± 31	38 ± 5	174 ± 33	38 ± 4
10	2	3	202	18	139	22
15	3	6	57	52 ± 16	52	64 ± 24
30	1	3	116	50	107	90
60	1	2	78	16	123	
360	1	1	45	45	69	

5 minutes. The 10-second values greater than 100 per cent presumably indicate incomplete mixing of endotoxin with circulating blood. The few observations made after 8 minutes (Table III) suggest that there is no significant recirculation of label for at least 6 hours following injection.

The curves in Figure 2 suggest that labeled endotoxin is cleared more rapidly from the blood of tolerant than of nontolerant rabbits. To determine whether a significant difference exists between the two groups, the data for whole blood clearance was analyzed statistically as follows:

A curve of the form

$$y_i = \alpha e^{(\beta_1 t_i + \beta_2 t_i^2)},$$

where y_i = cpm per ml of blood, t_i = time in minutes since injection of Cr^{51} endotoxin, e = base of the natural logarithms, and α, β_1, and β_2 are parameters to be estimated, was fitted to the data by the method of least squares. To facilitate fitting the model, the data were transformed by taking the natural logarithm of the counts per minute. The model then becomes

$$log_e y_i = log_e \alpha + \beta_1 t_i + \beta_2 t_i^2,$$

which was the form used. The deviations of the means of the transformed data from the fitted curves are not statistically significant ($p > 0.05$) when tested by the F statistic.

The estimated lines are:

Nontolerant: $y_i = 2957e^{-0.62686 t_i + 0.02760 t_i^2}$

Tolerant: $y_i = 2732e^{-1.03321 t_i + 0.016828 t_i^2}$

These curves and their 95 per cent confidence bands are plotted in Figure 3. The fact that the bands do not overlap except at their origins demonstrates that the curves are different. This was verified by testing the equality of the parameters, except α, for the two lines. The value of α is the best estimate of the level of radioactivity *immediately after injection* for the two groups. A t test for equality of levels of radioactivity at this point

in time gave a value of 0.5, demonstrating that the difference between these levels may be due to random variation among the animals in the two groups.

Clearance of Cr^{51} chloride

To determine whether these data reflect the clearance of endotoxin or Cr^{51} eluted *in vivo*, $Cr^{51}Cl_3$ was given to each of four nontolerant and three tolerant rabbits. The amount of chromium injected was equivalent to that present in 4 μg of Cr^{51} endotoxin preparation 3 (Table I) times the body weight in kg. The percentages of the dose in the circulating blood and plasma during the first 5 minutes after injection are shown in Figure 4. Clearance of $Cr^{51}Cl_3$ is distinctly slower than clearance of labeled endotoxin. It is of interest that tolerant animals did not clear $Cr^{51}Cl_3$ more rapidly than did nontolerant animals.

The demonstrated *in vitro* stability of the chromium^{51}-endotoxin bond and this difference in clearance of Cr^{51} endotoxin and $Cr^{51}Cl_3$ suggest that significant elution of the label from endotoxin does not occur *in vivo*. Braude and associates have reported similar findings and have shown

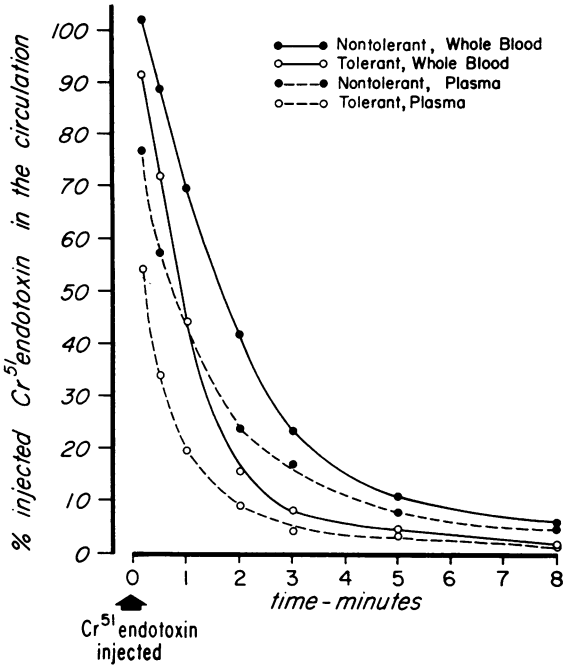

FIG. 2. CLEARANCE OF Cr^{51} ENDOTOXIN. Each point is the mean of 4 to 11 values.

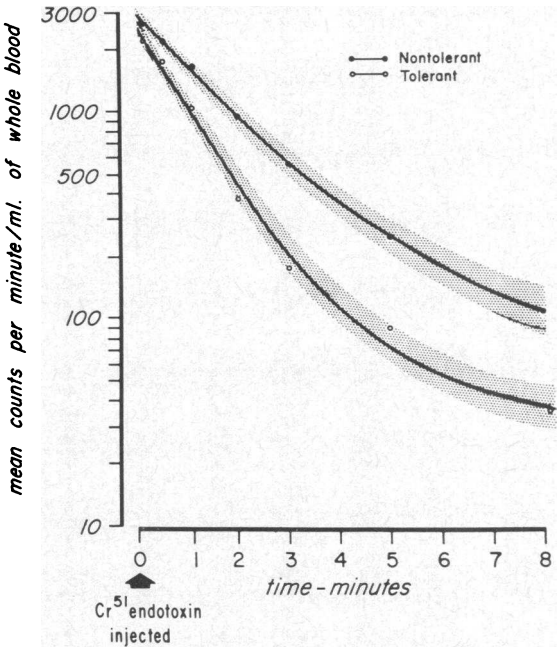

FIG. 3. CLEARANCE OF Cr^{51} ENDOTOXIN. The curves represent predicted geometric means of counts per minute per ml of whole blood at any point in time from 0 to 8 minutes after injection of Cr^{51} endotoxin. The points shown are geometric means of the observed counts per minute per ml of whole blood from 10 seconds to 8 minutes after injection. All points are within 95 per cent confidence bounds as shown.

further that the presence of unlabeled endotoxin does not alter chromium^{51} clearance (6, 8).

Distribution of Cr^{51}-labeled endotoxin in formed elements

Red cells. No radioactivity was detected in erythrocytes obtained from both nontolerant and tolerant animals at intervals of 30 seconds to 15 minutes after injection of Cr^{51} endotoxin.

Leukocytes. Lymphocytes, separated by eluting blood from cotton columns, were free of platelets and contained no radioactivity. Samples were obtained from nontolerant and tolerant animals within 1 to 2 minutes after Cr^{51} endotoxin injection; whole blood activity was high at this time.

Table IV shows the radioactivity in leukocytes obtained from whole blood by the dextran sedimentation technique and, for comparison, the radioactivity present in all formed elements of the blood from which the leukocytes were isolated. It is evident that only small amounts of radioac-

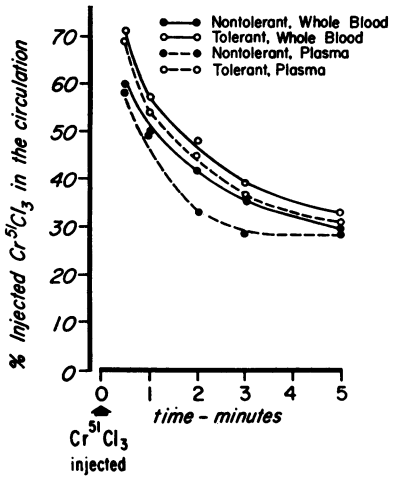

FIG. 4. CLEARANCE OF $Cr^{51}Cl_3$. Each point is the mean of two values except the 3- and 5-minute points in the nontolerant group, which are single values.

tivity, averaging less than 3 per cent of the radioactivity of all formed elements, are present in leukocytes isolated by this technique. The platelets isolated from the same samples all contained large amounts of radioactivity. Since red cells contained no radioactivity, at least 97 per cent of that in formed elements (Table IV) must be associated with platelets. Although the presence of minute amounts of Cr^{51} endotoxin in leukocytes cannot be excluded, the small amounts of radioactivity found may be readily explained by contamination with a small number of platelets; 1.2 to 6.5 per cent of the radioactivity of labeled platelets remain in the leukocytes separated by this technique (13, 15).

TABLE IV

Specific activity of leukocytes isolated from blood after injection of Cr^{51} endotoxin

Time after injection	Rabbits	Mean cpm/ml blood ±SE	
		In formed elements	In leukocytes
min	*no.*		
1	4	628 ± 19	13 ± 3.7
5	4	84 ± 8	3 ± 0.8

Table V presents the results of studies of localization of radioactivity in blood elements separated by silicone flotation. Reconstituted blood contains all the red cells, plasma, and most of the leukocytes, but less than 3 per cent of the platelets present in the original blood sample (Table II). The radioactivity of reconstituted blood is shown in column D of Table V. These values should be compared with those for the plasma in the same samples, column C, which also represents the activity of plasma present in whole blood. It is apparent that for at least 8 minutes after injection of labeled endotoxin the activity of reconstituted blood is the same as the activity contributed by plasma to either whole or reconstituted blood in both nontolerant and tolerant animals.

TABLE V

Specific activity of blood elements after injection of Cr^{51} endotoxin

Time after Cr^{51} endotoxin injection	State of rabbit	Samples	Mean counts per minute ±standard error						
			A Whole blood, 1 ml	B Plasma, 1 ml	C Plasma 1 ml in blood	D Reconst. blood, 1 ml	E All cells in 1 ml blood	F Platelets in 1 ml blood	G Ratio E:F
min		*no.*							
1	Nontolerant	3	1768 ±151	1593 ±121	973 ±33	974 ±40	795 ±126	794 ±124	1.001 ±0.009
	Tolerant	2	961	736	479	530	482	431	1.118
3	Nontolerant	4	555 ±46	615 ±87	391 ±46	392 ±43	164 ± 8	163 ± 3	1.033 ±0.039
	Tolerant	3	215 ±62	185 ±68	115 ±41	114 ±37	100 ±21	101 ±20	0.990 ±0.034
5	Nontolerant	3	323 ±89	360 ±102	233 ±69	236 ±60	90 ±25	87 ±32	1.153 ±0.204
	Tolerant	3	107 ± 6	87 ±11	57 ± 8	54 ± 7	50 ± 6	53 ± 8	0.956 ±0.030
8	Nontolerant	3	224 ±51	266 ±75	174 ±30	161 ±25	50 ±21	63 ±27	0.795 ±0.011
	Tolerant	4	42 ± 4	40 ± 3	26 ± 2	23 ± 2	16 ± 3	19 ± 3	0.798 ±0.060

Whole blood activity less that activity contributed by the plasma represents the activity contained in all formed elements of the sample (column E). The activity of whole blood minus the activity of reconstituted blood represents activity contained in platelets (column F). A comparison of columns E and F reveals that the activity of platelets is the same as that of all the formed elements. This is confirmed by the ratio of these values for each sample which approximates unity (column G).

DISCUSSION

This study has shown that Cr^{51} endotoxin given intravenously in small doses is cleared rapidly from the circulation, more rapidly in tolerant than in nontolerant animals. Carey, Braude, and Zalesky reported a similar difference in the rates of clearance of massive doses of Cr^{51} endotoxin; with smaller doses they observed a slightly greater rate of clearance in tolerant animals, but concluded the difference was not significant (7).

The results of studies of clearance of labeled endotoxin support earlier work using bioassay methods (4, 16). This agreement provides additional evidence for the validity of the use of chromium^{51} as an endotoxin label.

This study provides no evidence concerning the fate of endotoxin removed from blood or the mechanisms whereby clearance is increased in tolerant animals. The rapid uptake by the liver of Cr^{51} endotoxin (7) suggests a more or less direct transfer from blood to reticuloendothelial cells. This is supported by autoradiographic (17) and fluorescent antibody studies (18) demonstrating the rapid localization of endotoxin in reticuloendothelial cells. Beeson observed that injection of thorotrast into tolerant rabbits resulted in decreased clearance of typhoid vaccine as manifested by increased pyrogenicity of 4-minute serum (4). Since thorotrast is known to be engulfed by reticuloendothelial cells (17–19), the more rapid clearance in tolerant animals has been attributed to an increased rate of removal by fixed phagocytic cells (4). On the basis of studies in mice, Jenkin and Rowley suggest that "reticuloendothelial blockade" results from depletion of phagocytosis-promoting factors in blood rather than saturation of phagocytic cells (20). Conversely, the increased rate of clearance in tolerant animals may be due to higher blood levels of phagocytosis-promoting factors induced by previous exposure to endotoxin.

Mechanisms other than removal by fixed phagocytes possibly contribute to endotoxin clearance in tolerant animals. Large amounts of radioactivity have been found in the lungs of tolerant animals with high precipitin titers after injection of Cr^{51} endotoxin; this was not observed when precipitin titers were low (7). It was postulated that this represented rapid removal of endotoxin-precipitin complexes by the lungs.

This study indicates that most if not all endotoxin in formed elements of blood is associated with platelets. Carey and co-workers showed large amounts of radioactivity in the buffy coat after injecting Cr^{51} endotoxin (7). They did not report studies of its distribution among the constituents of buffy coat. Doering and Fritze found radioactivity in leukocyte and red cell fractions of human blood incubated with Cr^{51} endotoxin (21). Their method, however, did not provide for the separation of platelets from these fractions. In this study, the leukocytes isolated by the dextran sedimentation technique contained, on the average, only about 3 per cent of the activity of all formed elements at 1 and 5 minutes after injection (Table IV) when averages of 45 per cent and 28 per cent, respectively, of the whole blood radioactivity were present in the formed elements (Table V). Since this separation technique leaves 1.2 to 6.5 per cent of the platelets with the leukocytes (13, 15), it seems likely that the radioactivity found in these leukocytes resulted from platelet contamination. No radioactivity was found in leukocytes isolated by the silicone method. If endotoxin altered the specific gravity of leukocytes, any such cells lost in dextran sedimentation would have been recovered by the silicone technique. Since no activity was demonstrated in leukocytes recovered by the silicone method, it seems unlikely that preferential loss occurred in either system.

Almost complete separation of leukocytes and platelets was achieved with silicone flotation. The red cell-leukocyte layer (Figure 1) contained no significant radioactivity at any time following injection of Cr^{51} endotoxin. All of the activity present in the formed elements appeared to be in the platelet fraction. The alternate possibility that silicone induced transfer of radioactivity from

granulocytes to platelets *in vitro* seems highly improbable.

The absence of radioactivity from leukocytes recovered as described does not exclude uptake of some Cr^{51} endotoxin by granulocytes which promptly marginate (22) and are therefore not present in the blood samples. If leukocytes acquire labeled endotoxin and rapidly marginate, they must be immediately replaced by cells from the marginated or extravascular pools, since the concentration of labeled endotoxin falls much more rapidly than the concentration of leukocytes. Moreover, in tolerant animals, in whom clearance of endotoxin is even more rapid, marked changes in concentration of circulating leukocytes are not observed (Table II).

Rapidly developing thrombocytopenia following large intravenous doses of endotoxin has been reported to occur in rabbits (23–25) and in dogs (26). Engorgement of pulmonary capillaries with masses of platelets and leukocytes was observed as early as 10 minutes after injection (23). Marked alterations in numbers of circulating platelets were not observed after injection of the small doses of endotoxin used in this study. It is possible that immediate exchange of labeled for unlabeled platelets occurs, but the same considerations noted in regard to leukocytes probably apply also to platelets.

Davis, Meeker, and McQuarrie have shown that within 1 minute after injection of lethal doses of endotoxin into dogs, platelets undergo marked pseudopod formation, fusion, and viscous metamorphosis (26). Platelet aggregation with release of serotonin, phospholipid, and bactericidins active against *B. subtilis* has been demonstrated on incubation of platelet-rich rabbit plasma with endotoxin (27). Endotoxin-induced platelet agglutination and serotonin release have also been reported to occur *in vivo* in rabbits (28, 24). Horowitz, Des Prez, and Hook have reported activation of platelet factor 3 in the blood of rabbits immediately after intravenous injection of endotoxin (29). In view of these findings, it is likely that some of the biologic effects of endotoxin result from the action of substances released from platelets.

Good evidence exists that the febrile response to endotoxin is mediated by a pyrogen derived from leukocytes (30–33). The disappearance of small doses of endotoxin from the circulation before onset of fever and leukopenia and the absence of injected endotoxin from circulating leukocytes suggest that leukopenia and release of endogenous pyrogen also are produced by indirect mechanisms. Initial studies of reserpine-treated rabbits show that although fever is inhibited, the leukopenic response to endotoxin is unaffected (34). This suggests that the release of serotonin from platelets is not directly responsible for leukopenia.

Until the mechanisms of production of endotoxin effects are better understood, the relationship between accelerated clearance of endotoxin and the altered biologic responses in tolerant animals is conjectural.

SUMMARY

Blood and plasma clearance of small doses of Cr^{51}-labeled endotoxin was more rapid in tolerant than in nontolerant rabbits. In both groups, clearance was nearly complete within 10 minutes after injection. Circulating endotoxin was distributed between plasma and platelets in both nontolerant and tolerant animals. The minute amounts of endotoxin found in leukocytes could have been due to contaminating platelets.

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