

# THE DISTRIBUTION OF CR<sup>51</sup> LABELED *E. COLI* ENDOTOXIN IN THE GENERALIZED SHWARTZMAN REACTION<sup>1</sup>

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The generalized Schwartzman reaction (1) occurs in immature (2) rabbits following the second of two sublethal intravenous injections of gram-negative bacterial endotoxin, spaced 18 to 24 hours apart, and consists of widespread hemorrhage and necrosis. The reaction also occurs after a single injection of endotoxin in animals treated with intravenous thorotrast, trypan blue (3), colloidal iron, or colloidal carbon (4). Because these substances were thought to "blockade" the reticuloendothelial (R.E.) system, it was suggested that an analogous impairment of R.E. function may also be produced by the first or "preparing" injection of endotoxin, resulting in slower removal and detoxification of the second dose and directly or indirectly in the characteristic lesions in the kidney, heart, lungs, and liver.

In the experiments to be reported, the distribution of radioactive Cr<sup>51</sup> labeled endotoxin in normal rabbits was compared with that in rabbits which had received a preparing injection of unlabeled endotoxin 24 hours earlier. The results indicate that, in the rabbit, the "prepared" state is characterized by impaired removal of a second injection of endotoxin by the liver and increased localization by the lung.

## METHODS AND PROCEDURE

**Animals.** Young albino rabbits of a hybrid stock weighing between 0.77 and 1.3 kilo., used for all experi-

ments, were housed in air-conditioned, humidity-controlled animal quarters and fed water and Purina rabbit pellets.

**Endotoxin.** *Escherichia coli* endotoxin was prepared and labeled with radioactive chromic chloride (Cr<sup>51</sup>Cl<sub>3</sub>) according to the methods previously reported (5). The close quantitative relationship shown to exist between the toxicity and radioactivity of endotoxin so labeled provides the basis for using radioactivity as a measure of the distribution of endotoxin. In preliminary experiments it was found that the lot of endotoxin employed in these experiments produced the generalized Schwartzman reaction with high frequency in immature rabbits.

**Experimental.** Each rabbit in one experimental group received an intravenous injection of 0.25 mgm. non-labeled *E. coli* endotoxin in the marginal vein of the ear (the "preparing" injection); 24 hours later each prepared animal received a second intravenous injection of 0.56 mgm. *E. coli* endotoxin labeled with Cr<sup>51</sup>Cl<sub>3</sub>. In a second group of animals, each received an intravenous injection of 0.56 mgm. labeled endotoxin, but no preparing injection of non-labeled endotoxin.

At varying intervals after the injection of radioactive endotoxin, samples of blood were removed by cardiac puncture, heparinized, and 5.0 ml. plasma saved for determination of radioactivity. The animals were immediately sacrificed and the weights of each animal and its individual organs were recorded. The lung, heart, spleen, kidney, and weighed aliquots of liver were rinsed with tap water to remove superficial blood and placed in 10 per cent formol-saline until radioactivity was measured. After counting, sections of the organs, stained with hematoxylin and eosin were examined microscopically. When the volume of an organ made it necessary to digest with concentrated nitric acid before counting, a weighed aliquot was first removed for histologic section. The radioactivity of the tissues and of injected labeled endotoxin was determined by counting in a scintillation counter for ten minutes as previously described (5). Corrections were made for decay. On the basis of previous data obtained in rabbits given endotoxin, total circulating plasma radioactivity was calculated by assuming an average hematocrit of 40 per cent and an average blood volume of 5 per cent of body weight (6).

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TABLE I  
Comparison of uptake of radioactive Cr<sup>51</sup> labeled endotoxin in normal rabbits and those which received non-labeled endotoxin twenty-four hours earlier

Rabbit number	Interval between injection and sacrifice	% Injected radioactivity*						Concentration index†				
		Plasma 5 ml.	Spleen	Liver	Lung	Heart	Kidney	Spleen	Liver	Lung	Heart	Kidney
Group I—0.25 mgm. <i>E. coli</i> endotoxin, I. V. 24 hours prior to 0.56 mgm. Cr <sup>51</sup> labeled endotoxin I. V.												
16	35'	3.8	1.2	41.7	3.0	0.2		17.1	7.1	4.3		
†17	38'	5.8	2.7	21.9	4.0	0.5	3.6	13.0	6.2	3.5	2.1	2.3
†18	75'	3.5	4.5	28.1		0.3	1.8	20.3	6.1		1.7	1.0
†20	77'	3.9	1.3	21.7		0.2	0.6	16.4	5.6		1.1	0.5
21	123'	2.4	1.3	49.7	1.7	0.2	0.7	21.8	11.2	2.8	1.2	0.8
†22	125'	2.7	4.7	25.7	4.4	0.4	2.0	27.4	10.3	5.4	1.7	1.5
†23	180'	3.5	1.6	31.9	2.3	0.1	1.9	19.5	8.5	3.1	0.7	1.9
24	185'	2.7	6.5	24.4	6.1	0.3	0.3	49.9	5.8	7.2	1.2	0.3
†25	255'	5.1	1.3	30.6			0.3	14.6	7.3			3.0
26	257'	2.3	2.0	29.4	2.5	0.3	0.4	22.9	6.75	3.2	1.5	0.4
27	363'	1.4	13.7	29.3	3.2	0.2	0.5	57.1	7.0	3.4		
†28	364'	2.6	2.9	40.3	6.0	0.1	1.3	29.0	9.3	7.0	0.4	1.1
§30	11°	0.7	1.9		4.0	0.1	3.9	15.8		4.1	0.4	3.2
†31	11°	2.6	1.3		1.4	0.4	0.4	11.0		1.7	1.9	0.4
†32	18°	1.2	2.4	34.4	2.4	0.1	0.9	11.9	8.5	3.4	0.8	1.1
§33	24°	0.5	3.6		2.1	0.3	2.1	23.8		2.3	1.4	2.0
†34	24°	0.9	5.6	28.9		0.1	0.4	5.1	8.6			
§35	15°		6.9	27.9	2.8	0.4	7.0	36.5	5.3	2.5	1.8	4.8
Group II—Single injection I. V. 0.56 mgm. Cr <sup>51</sup> labeled endotoxin												
36	41'	3.1	2.1	46.9		0.3	1.7	38.5	8.3		1.7	2.2
†37	43'	4.4	3.6	42.6	0.2	0.3	1.4	36.2	15.9	0.4	1.7	1.9
38	79'	5.3		46.9	0.6	0.4	0.6		7.4	1.3	1.9	0.7
39	81'	5.0	3.6	57.5	0.8	0.4	1.2	29.8	10.8	1.1	2.2	1.5
40	128'	1.4	2.6	68.1	1.9	0.2	0.4	28.3	14.7	3.3	0.9	0.4
†41	130'	4.0	1.9	57.5	0.5	0.4	0.8	28.4	10.4	0.8	1.4	0.8
42	190'	2.8	2.5	53.1	3.2	0.3	1.7	13.9	13.4	5.0	1.2	1.0
†43	192'	4.0	1.1	45.6	2.1	0.2	1.5	15.4	13.4	3.0	0.9	1.7
44	262'		0.9	31.9	3.2	0.2	0.3	5.5	10.2	4.9	1.3	0.4
45	264'	3.1	2.4	58.6	1.9	0.2	1.0	26.1	17.9	2.4	1.1	1.2
46	367'		1.6	57.5		0.1	0.8	31.5	11.7		0.4	0.8
†47	369'	3.0	2.0	47.4	1.9	0.3	0.3	28.3	8.9	2.6		
§48	11°	2.4	2.3		0.9	0.5	1.9	38.9		1.6	1.9	1.8
49	11°	2.5	2.0	50.4	1.1	0.2	0.5	25.8	12.2	1.8	0.7	0.4
50	18°	1.1	2.7	54.4	0.8	0.3	0.1	37.5	13.0	1.2		
51	18°	1.7	1.4	41.1	1.9	0.2	1.4	18.2	10.6	0.6		1.7
52	24°	0.9	1.9	67.7		0.2	0.2	25.8	19.2			
53	24°	1.2	1.6	45.5	0.7	0.4	1.1	27.2	11.9	0.9	2.0	1.7

\*  $\frac{\text{c.p.s. in total organ}}{\text{c.p.s. injected}} \times 100 = \% \text{ Injected radioactivity in given organ.}$

†  $\frac{\text{Per cent of injected radioactivity in given organ}}{\text{Per cent of total body weight in organ}} = \text{Concentration index.}$

‡ Fibrinoid deposits in glomeruli but no necrosis detectable microscopically.

§ Bilateral renal cortical necrosis present grossly and microscopically.

## RESULTS

The results of these studies are given in detail in Table I and presented graphically in Figures 1, 2, 3, and 4.

The distribution of radioactive endotoxin in the normal rabbits resembles that reported previously (6). Figure 1 shows that clearance of radioactivity from the plasma of the prepared animals did not differ significantly from normals.

On the other hand, uptake of radioactivity by the liver (Figure 2) was strikingly reduced in the prepared animal. Hepatic uptake accounted for as much as 60 per cent of injected radioactivity in the normal rabbit, whereas uptake in the prepared rabbit was usually less than 40 per cent. Figure 4 shows that the lung of the prepared rabbit accumulates a higher percentage of the injected radioactivity than the normals.

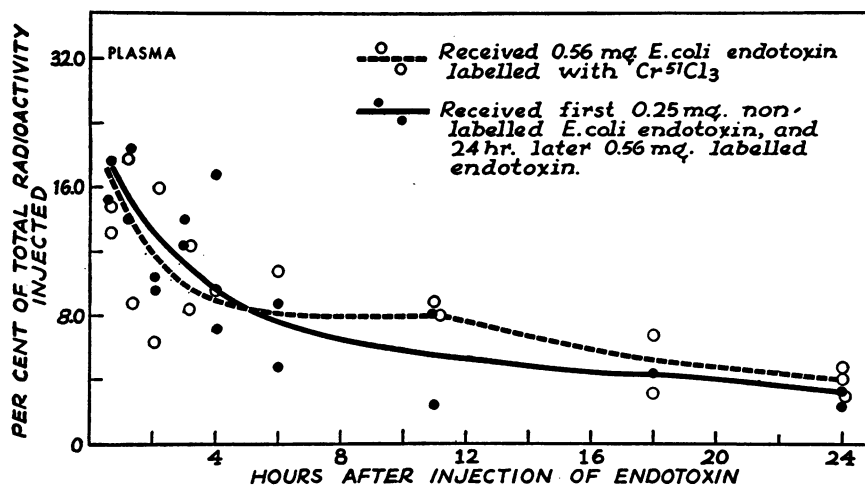


FIG. 1. PER CENT OF INJECTED RADIOACTIVITY PRESENT IN PLASMA AT VARIOUS TIMES AFTER INTRAVENOUS INJECTION OF  $\text{Cr}^{51}$  LABELED ENDOTOXIN, IN NORMAL RABBITS AND ANIMALS WHICH HAD RECEIVED A PREPARING INJECTION OF 0.25 MGM. UNLABELED ENDOTOXIN 24 HOURS EARLIER

Each point represents the value for a single animal.

Uptake of radioactive endotoxin by the kidneys in the two groups did not differ significantly except in the rabbits which developed bilateral renal cortical necrosis (Figure 3). The kidneys of four such animals contained an average of 3.72 per cent of radioactivity injected, whereas the kidneys of those living more than 6 hours and not showing renal cortical necrosis contained an average of only 0.62 per cent. This apparent increase in uptake of radioactivity is not explained simply by the increased size of the involved kidneys, because the average concentration index was also increased in that group. Excluding those developing necrosis, glomerular fibrinoid deposits were found in the kidneys of 5 single-dose rabbits, but in 13 prepared animals. In contrast, no significant differences in localization of radioactivity by these two groups were observed.

Bilateral renal cortical necrosis occurred with the expected incidence (3 of 6 living more than 6 hours) in the prepared group, but one animal also showed this lesion after one injection of endotoxin. Presumably, the fibrinoid deposits occluding the glomerular lumen as a result of a single injection of endotoxin were sufficiently extensive to result in cortical necrosis (7).

Radioactivity in the spleen in the two groups varied considerably, but the mean values were slightly higher for the prepared group than for

the controls. (Mean uptake in prepared group equalled 3.63 per cent, in controls, 2.11 per cent.)

Not all of the injected radioactive endotoxin was accounted for in the organs examined and it seems possible that localization may have occurred in the bone or other points in the carcass not examined. On the basis of previous data (6), intestinal or renal excretion probably would not account for a significant amount of endotoxin.

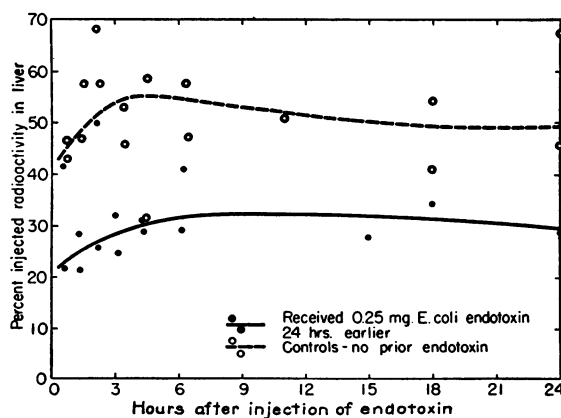
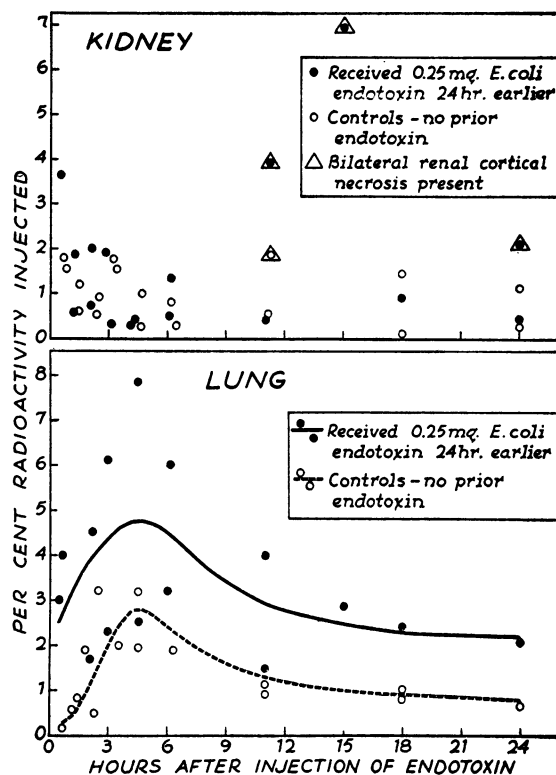


FIG. 2. PER CENT OF INJECTED RADIOACTIVITY LOCALIZED IN THE LIVER AT VARIOUS TIMES AFTER AN INTRAVENOUS INJECTION OF  $\text{Cr}^{51}$  LABELED ENDOTOXIN IN NORMAL AND PREPARED RABBITS

Each point represents the value for a single animal.



FIGS. 3, 4. PER CENT OF INJECTED RADIOACTIVITY IN THE KIDNEY (FIGURE 3) AND LUNG (FIGURE 4) AT VARIOUS TIMES AFTER AN INTRAVENOUS INJECTION OF  $\text{Cr}^{51}$  LABELED ENDOTOXIN IN NORMAL AND PREPARED RABBITS

Each point represents the value for one animal.

#### DISCUSSION

These experiments demonstrate significant alterations in localization of endotoxin in the organs of the rabbits prepared for the generalized Shwartzman reaction. Twenty-four hours after a sublethal intravenous injection of *E. coli* endotoxin, the capacity of the liver to take up radioactive  $\text{Cr}^{51}\text{Cl}_3$  labeled endotoxin was reduced to approximately one-half normal. On the other hand, the uptake by the lung, and to a lesser extent by the spleen, was increased in the prepared rabbits. The relation of these shifts in localization of radioactive endotoxin to pathogenesis of the generalized Shwartzman reaction, however, is not clear. In general, prepared rabbits showed the altered pattern of localization regardless of whether or not renal cortical necrosis developed. Likewise, rabbits having glomerular fibrinoid lesions upon microscopic examination did not differ consist-

ently in pulmonary or hepatic uptake from those not showing this lesion.

The only definite correlation between shifts of radioactivity and the lesions of the generalized Shwartzman reaction was found in the increased localization in kidneys showing renal cortical necrosis. Although this observation implies that increased localization of endotoxin in the kidney may result directly in cortical necrosis, another possible explanation seems as likely. Previous studies (6) have demonstrated that soon after injection into normal rabbits radioactive endotoxin localized in the "buffy coat" of the blood and presumably in the polymorphonuclear leucocytes. Since renal cortical necrosis is accompanied by infiltration of the swollen juxta-cortical areas with leucocytes (8), it seems possible that renal concentration of radioactivity may represent the trapping of endotoxin-laden leucocytes in the necrotic areas. This same mechanism might also account for greater uptake by the lung.

The decreased uptake of endotoxin in the liver of the prepared rabbits is of considerable interest. The concept that R.E. function of the liver is impaired by the preparing injection of endotoxin receives support from several previous studies. "Blockade" of the R.E. system (3, 4) brings about a state of preparation for the generalized Shwartzman reaction in rabbits which resembles, in every demonstrable way, that resulting from an injection of endotoxin. Cornwell (9) determined plasma clearance rates and organ uptake of radioactive colloidal gold in prepared rabbits in an attempt to assess the functional capacity of the R.E. system after an injection of endotoxin. He demonstrated a decreased plasma clearance and hepatic uptake in the prepared rabbit. Gabrielli (10) has reported similar results using radioactive colloidal chromium, and Biozzi, Benacerraf, and Halpern (11) recently demonstrated decreased hepatic uptake of injected ink particles after injection of typhoid vaccine.

Therefore, the available evidence indicating decreased capacity of the liver to take up endotoxin is compatible with the interpretation that an R.E. function of this organ is compromised in the "prepared" rabbit. It is conceivable, however, in view of the marked hemodynamic effects of endotoxin, especially involving splanchnic and hepatic blood flow (12), that other mechanisms may be in-

volved. Regardless of their interpretation the functional alterations of the prepared state revealed in these studies do not appear to bear a direct relationship to the microscopic fibrinoid lesions which characterize the generalized Shwartzman reaction. In this respect the data support the suggestion made previously (13, 14) that the acute lethal action of endotoxin and the fibrinoid vascular lesions in the Shwartzman reaction do not necessarily depend upon the same mechanisms.

## SUMMARY

Attempts have been made to characterize the prepared state in the generalized Shwartzman reaction by determining the fate of intravenously injected  $\text{Cr}^{51}$  labeled radioactive endotoxin in rabbits 24 hours after a preparing injection of unlabeled endotoxin. Compared with normal rabbits, the prepared rabbit exhibited a markedly altered pattern of distribution of radioactivity. Hepatic uptake was reduced nearly 50 per cent and pulmonary uptake was increased. No increase in renal localization was observed except in those rabbits developing bilateral renal cortical necrosis. In animals not developing necrosis, no direct relationship was found between the renal or hepatic uptake of endotoxin and the microscopic fibrinoid lesions of the generalized Shwartzman reaction.

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