results support the proposed hypothesis that the renal and vascular lesions observed in diabetes are produced by a mechanism involving insulin derivatives, gamma globulin, and complement components and serve as a convenient model for further study.

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Bactericiadl Activity of Fixed Phagocytes in Irradiated and Unirradiated Mice Treated with Enodtoxin* (33591)

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Smith *et al.* (1) reported that a single injection of bacterial endotoxin given 24 hr before X-irradiation resulted in a significant increase in survival in mice. In a subsequent study, they showed that there was an increased resistance to experimental infection and more rapid mobilization of granulocytes in peripheral blood (2) due to an earlier recovery of the cellular elements in bone marrow (3). Ainsworth and Hatch (4) confirmed these studies and also found a significantly lower incidence of endogenous infection in mice treated with endotoxin. Savage (5) showed that endotoxin did not prevent damage to bone marrow and spleen in mice but hastened their recovery. The bone marrow of endotoxin-treated mice and controls looked the same histologically for the first 3 days after X-irradiation. On the fifth day foci of neutrophils were observed but recovery was not complete until days 17–18. Without streptomycin treatment, controls were dead by day 12. Perkins *et al.* (6) found a significant increase in intracellular digestion of chicken erythrocytes by peritoneal phagocytes obtained from irradiated mice treated with endotoxin. They were unable, however, to demonstrate that endotoxin increased the survival of irradiated mice.

It had previously been established that death in animals receiving 600–800 R X-irradiation was due to leukopenia, hemorrhage, anemia and infection (7). Bacteremia in irradiated animals was reported to be due to the inability of phagocytes to destroy ingested microorganisms rather than an inability to ingest bacteria (8).

The experiments reported here were undertaken to determine whether or not the lower

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mortality among irradiated mice injected with endotoxin might not be due partly to an increased ability of phagocytes in liver and spleen to kill ingested bacteria. This seemed a feasible explanation since the number of granulocytes in the blood stream during the first week post irradiation is quite low. Endotoxin has been shown to enhance the phagocytic capacity of the reticuloendothelial system in normal animals (9). It has been shown also to cause increased nonspecific resistance to infection (10) which has been attributed to a higher opsonic activity of the serum (11). It was of interest to determine whether or not endotoxin would have any effect on the bactericidal ability of fixed phagocytes in unirradiated mice.

Materials and Methods. Mice. CF1 females 10–14 weeks old were kept in an air-conditioned, humidity controlled room and supplied with food and water *ad libitum.* They were housed in large stainless steel cages which were changed twice weekly. Water bottles were changed daily.

Endotoxin. The lipopolysaccharide from the cell walls of *Salmonella typhosa,* control 111019, obtained from Difco Laboratories, Inc., Detroit, Michigan, was dissolved in sterile physiological saline. The same stock solution was used in all experiments. It was kept frozen and was thawed immediately prior to use so that fresh dilutions were made for each experiment in physiological saline.

Irradiation.[1] The total dose of 550 R was delivered by a General Electric Maximar 400 using 400 kV, 5 mA, inherent filtration 1.0 mm Al and added filtration 0.2 mm Cu. The target distance was 50 cm and the dose rate was approximately 80 R/min. For irradiation the mice were placed into a round plastic perforated cage with 10 wedge-shaped compartments. The cage, mounted on a turntable centered directly under the X-ray beam, revolved slowly during exposure.

Challenge microorganism. A strain of *Pseudomonas aeruginosa* highly resistant to streptomycin was used as the challenge microorganism. Its virulence was maintained by weekly passage through unirradiated CF_1 mice. An 18-hr culture on an agar (containing 1000 μ/ml streptomycin) plate was suspended in 5 ml of physiological saline. After clumps of bacteria were broken up the suspension was diluted to approximately 10^9 microorganisms/ml by means of a Coleman spectrophotometer. Tenfold dilutions were made and the bacterial content checked by plating, in triplicate, 0.1 ml of the 10^{-6} and 10^{-7} dilutions.

Experimental procedure. All endotoxin injections were given 24 hr before irradiation. Four groups containing 4 mice each were used per experiment. One group received intravenously 3 μg of endotoxin in 0.5 ml of saline prior to 550 R of X-irradiation while a second group, the controls, received 0.5 ml of saline at the same time. A third group received 3 μg of endotoxin in 0.5 ml of saline and no irradiation while a fourth group, the controls, received 0.5 ml of saline. Four days after irradiation all animals were injected intravenously with approximately 2.8×10^7 *Ps. aeruginosa* in 0.5 ml of saline. Unirradiated mice were inoculated intravenously with approximately 2.2×10^8 pseudomonads on the same day. The pseudomonad injections were followed 1 min later by 5 mg of Colistin[2] to kill any extracellular microorganisms. Approximately 24 hr later the mice were killed by cervical fracture. The abdomen was opened and the liver and spleen were removed. Livers from each group of 4 mice were put into 5 ml of chilled trypticase soy broth (containing 1000 μ/ml of streptomycin) in a tissue grinder. Spleens were put into 2.5 ml of the same broth and homogenized by means of a motor driven Teflon grinder. Appropriate dilutions were made in chilled broth and 0.1 ml was plated in triplicate on nutrient agar containing streptomycin. The inocula were spread by means of glass beads and the plates were incubated at 37° for 24 hr. The plates which yielded between 30 to 300 colonies were counted. Heart's blood was obtained from each mouse by aspiration

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[2] Colistin was obtained from Warner-Chillcot Laboratories through the courtesy of Dr. Robert M. Gabrielson.

TABLE I. Numbers of *Ps. aeruginosa* Recovered in Cultures of Organs from X-Irradiated Mice Injected Intravenously with 3 μg of *S. typhosa* Endotoxin.

		No. of bacteria recovered/mean[a] organ			
	No. of *Ps. acruginosa* injected ($\times 10^7$)	Liver[b]		Spleen[b]	
		Saline controls	Endotoxin treated	Saline controls	Endotoxin treated
	1.90	2.42×10^5	875	426	24
	3.90	4.88×10^6	200	42	126
	2.20	1.79×10^2	116	6	2
	2.25	50	146	5	7
	2.15	2.39×10^5	160	94	554
	2.25	1.09×10^5	154	82	12
	2.20	4.10×10^5	6.96×10^5	72	45
	2.50	5.06×10^5	175	64	142
	4.15	1.18×10^6	1209	42	9
	3.10	2.69×10^4	166	40	9
	3.20	1.79×10^4	651	34	28
	3.50	1.76×10^4	1191	43	172
	2.60	1.11×10^4	125	19	7
	3.55	2.21×10^3	5.70×10^5	18	15
	2.15	2.76×10^4	6250	695	30
	2.50	3.34×10^4	1325	194	405
	2.95	6.75×10^4	165	94	48
	2.80	1.00×10^6	88	194	8
	4.65	1.90×10^4	1212	306	90
	1.60	1.75×10^2	34	14	8
Mean	2.80	2.20×10^5	6.41×10^4	124	87
Difference		1.56×10^5		37	

[a] Each number represents the average of 4 organs.

[b] Statistical analysis using the rank test shows significant difference at the 1% level.

with a Wright's pipette. One drop was cultured and any experiments in which mice were found to have positive heart's blood were excluded. There were 320 mice in this set of experiments.

To test whether or not the route of administration of endotoxin made a difference an additional set of 5 experiments, 20 mice each, was carried out. The same protocol as above was followed except that 12 μg of endotoxin in 0.2 ml of saline were administered intraperitoneally.

Results and Discussion. Tables I and II summarize the results obtained with X-irradiated and unirradiated mice injected intravenously with 3 μg of endotoxin and their controls. Appreciable differences were found in bacterial counts in livers of endotoxin-treated animals as compared with those treated with saline. Although individual counts from experiment to experiment varied over a wide range the overall tread of the determinations indicated that fewer bacteria were killed in the livers of the controls. The average number of bacteria recovered in 20 experiments (160 mice) from livers of X-irradiated mice was 2.20×10^5 whereas 6.41×10^4/average liver were recovered from the X-irradiated endotoxin-treated animals. This indicated that the endotoxin-treated animals, on the average, destroyed 1.56×10^5 more bacteria/liver than the controls. The unirradiated endotoxin-treated animals destroyed 1.84×10^6 more bacteria/liver than their controls.

The spleens of the unirradiated animals treated with endotoxin showed an enhanced bactericidal ability over controls. However,

TABLE II. Numbers of *Ps. aeruginosa* Recovered in Cultures of Organs from Unirradiated Mice Injected Intravenously with 3 μg of *S. typhosa* Endotoxin.

		No. of bacteria recovered/mean[a] organ			
		Liver[b]		Spleen[b]	
	No. of *Ps. aeruginosa* injected	Saline controls	Endotoxin treated	Saline controls	Endotoxin treated
	1.90×10^8	5.19×10^5	5.00×10^3	210	304
	3.90×10^8	7.71×10^6	2.90×10^2	114	124
	2.20×10^8	5.79×10^6	2.76×10^3	317	216
	2.25×10^8	3.50×10^5	1.56×10^5	154	110
	2.15×10^8	6.46×10^5	7.66×10^5	200	152
	2.25×10^8	5.35×10^5	8.00×10^4	757	332
	2.20×10^8	3.97×10^5	6.25×10^5	1054	460
	2.50×10^8	4.50×10^6	3.12×10^5	4248	310
	4.15×10^8	7.62×10^6	2.62×10^3	350	298
	3.10×10^8	2.32×10^6	1.58×10^3	710	432
	3.20×10^8	1.45×10^6	1.09×10^3	524	436
	3.50×10^8	2.06×10^6	2.48×10^3	1786	1650
	2.60×10^8	3.52×10^6	1.36×10^5	160	484
	3.55×10^8	3.86×10^5	1.85×10^6	2866	2516
	2.15×10^8	2.95×10^6	1.71×10^6	1500	334
	2.50×10^7	3.36×10^4	8.21×10^2	52	44
	2.95×10^7	3.66×10^4	1.79×10^2	822	346
	2.80×10^7	6.96×10^5	71	68	34
	4.65×10^7	9.37×10^5	1.59×10^2	102	90
	1.60×10^7	1.88×10^2	96	206	520
Mean	2.15×10^8	2.12×10^6	2.82×10^5	810	460
Difference		1.84×10^6		350	

[a] Each number represents the average of 4 organs.
[b] Statistical analysis using the rank test shows significant difference at the 1% level.

no such difference was observed between the spleens of endotoxin-treated animals and controls in the irradiated groups. This lack of difference may be due to the extremely small size of the spleens in the irradiated mice.

In 5 experiments the challenging dose of bacteria was the same for all groups hence it was possible to compare directly endotoxin-treated X-irradiated mice with endotoxin-treated unirradiated mice. These data are summarized in Table III. It can be seen that no significant difference in bactericidal ability was obvious in these groups of animals although somewhat fewer bacteria were recovered from the livers of the latter. This indicates that the endotoxin-treated X-irradiated animals were almost as capable of destroying phagocytosed bacteria as endotoxin-treated unirradiated ones and certainly as capable of destroying phagocytosed bacteria as normal animals not receiving any endotoxin.

In the 5 experiments done to determine

TABLE III. Numbers of *Pseudomonas aeruginosa* Recovered in Cultures of Organs from Mice Injected Intravenously with 3 μg of *S. typhosa* Endotoxin.

	No. of *Ps. aeruginosa* injected ($\times 10^7$)	X-irradiated		Unirradiated	
		Liver	Spleen	Liver	Spleen
	2.50	1325	405	821	44
	2.95	165	48	179	346
	2.80	88	8	71	34
	4.65	1212	90	159	90
	1.60	34	8	96	520
Mean	2.90	565	112	265	207

whether or not the route of administration of endotoxin would affect the results, no difference was observed between the intravenous and the intraperitoneal routes.

The effects of endotoxin are known to last no longer than 7 days and are already on the decline on the fifth day in reticuloendothelial cells (9). However, it is at this time regeneration of neutrophils is first noted. One of the reasons irradiated mice treated with endotoxin survived may be that endotoxin by preserving the bactericidal ability of the Kupffer cells (and possibly other reticuloendothelial cells) prevented infection and allowed time for bone marrow regeneration. Endotoxin stimulates an earlier hematopoietic recovery hence granulocytes are available sooner should the reticuloendothelial cells require aid in combating an infection.

The increased bactericidal ability observed in the livers and spleens of unirradiated mice can be explained possibly by the fact that endotoxin stimulated an increase in the number of actively phagocytic cells. An increase in the number of actively phagocytic cells in the liver has been demonstrated following endotoxin administration (9, 12) although the authors do not agree as to the origin of these cells.

Studies on serum proteins (13) indicate that there is little increase in gamma globulins but a considerable increase in the beta-globulin fractions after endotoxin administration. The rise in serum opsonins is short-lived, returning to normal by the seventh day. Although serum factors cannot be ignored as contributing to the increased resistance to infection in endotoxin-treated animals, it is not likely that they contributed very much to the results of this investigation. The challenge by pseudomonads was on the fifth day after endotoxin administration when the effect on leukocytes and serum globulins in practically dissipated.

Summary. Mice were injected intravenously or intraperitoneally with 3 or 12 μg of *S. typhosa* endotoxin, respectively. One group received 550 R of X-irradiation 24 hr later. Five days after endotoxin administration all mice were injected intravenously with *Ps. aeruginosa*. One min after receiving the pseudomonad injection the mice were given 5 mg of Colistin intravenously to kill extracellular bacteria. Livers and spleens were removed from the infected mice 24 hr later, ground, and the number of viable bacteria per organ was determined by culture methods. Heart's blood was cultured for extracellular bacteria. The results indicated that greater numbers of bacteria were killed within the livers and spleens of endotoxin-treated animals than controls. Only the livers of endotoxin-treated X-irradiated animals showed higher bactericidal ability.

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