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US ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 565

ALTERED RENOPRESSOR RESPONSE-PATTERN TO ENDOTOXIN RADIATED WITH RADIO-FREQUENCY ENERGY

E. S. Dooley, Ph.D.
Capt J. Y. Gillenwater, MC
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UNITED STATES ARMY
MEDICAL RESEARCH AND DEVELOPMENT COMMAND

17 July 1963

Report Submitted 18 February 1963

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The animals used in this study were handled in accordance with the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

AD Accession No.

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2. S. Typhosa Endotoxin
3. Radio-Frequency Energy

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US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky

17 July 1963

Army Medical Basic Research in Life Sciences
DA Project No. 3A012501B811

USAMRL Report No. 565
DA Project No. 3A012501B813

ABSTRACT

ALTERED RENOPRESSOR RESPONSE-PATTERN TO ENDOTOXIN RADIATED WITH RADIO-FREQUENCY ENERGY

OBJECT

To determine if radio-frequency energy alters the pharmacodynamic properties of S. typhosa endotoxin.

RESULTS

Injected S. typhosa produces a characteristic diphasic pressor response in the renal vascular bed. There is an immediate transient mild rise followed by a more intense and prolonged rise which subsequently returns toward or to the pre-injection level. Radiating S. typhosa at 10.40 Mc/sec modified the above pressor response-pattern. The first pressor response was considerably enhanced and the second remained permanently sustained, i.e., there was no return toward the pre-injection pressure level. Radiating endotoxin at 13.34 Mc/sec intensified the first pressor response but did not modify the second pressor response.

RECOMMENDATIONS

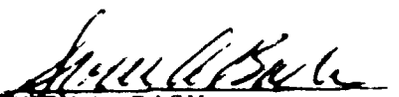
1. It is recommended that the nature of the change (physical, chemical?) induced in the S. typhosa material by radiation with 10.40 Mc/sec be investigated.

2. It is recommended that it be determined whether radiation of this material alters its lethal characteristics by using an appropriately designed experimental series with small animals such as cockerels or mice.

3. The effect of other radio-frequency energies should be similarly investigated.

APPROVED: 

SVEN A. BACH
Colonel, MC
Director, Biophysics Division

APPROVED: 

SVEN A. BACH
Colonel, MC
Director

ALTERED RENOPRESSOR RESPONSE-PATTERN TO ENDOTOXIN RADIATED WITH RADIO-FREQUENCY ENERGY

I. INTRODUCTION

The investigation of the effects of high-frequency currents on bacterial toxins was first studied shortly after the work of Tesla in 1891 (1). D'Arsonval (2) treated diphtheria exotoxin with short-waves and reported that this treatment resulted in a diminution of toxicity. Szymanoski (3) reported that ultrahigh-frequency radiation is capable of producing definite attenuation of the three major bacterial toxins; diphtheria, tetanus, and botulinus, in raw broth filtrates. This effect was obtained without the development in the toxin of temperatures that would by themselves affect the potency of the toxin. There has been little work done on the effects of radio-frequency energy on bacterial endotoxins.

It has been reported by Bach et al (4) that the electrophoretic pattern and antigenic reactivity of human gamma globulin can be altered by exposing the protein in vitro to radio-frequency energy (R.F.E.) of the proper frequency and field strength. A more recent investigation indicated that R.F.E. irradiation may also alter other protein systems, such as serum amylase (5).

A purified lipopolysaccharide complex of S. typhosa was chosen for this investigation primarily because it is exceptionally thermostable (resists autoclaving and boiling) (6-8).

II. MATERIALS AND METHODS

Animals. Dogs, weighing 10-15 kg, were used and anesthetized with intravenously administered sodium pentobarbital (33 mg/kg). Heparin sodium (5 mg/kg) was administered intravenously.

Renal Perfusion. The kidney was perfused by the technique of Hardin et al (9). In brief, the abdominal aorta was exposed retroperitoneally through a left flank incision and the lumbar vessels tied. Blood from the body pool was then withdrawn from a carotid artery with a precalibrated pressure-independent Sigmamotor pump (Model T-6) and perfused into the abdominal aorta in a cephalad direction through a right angle glass cannula tied in position 3 cm below the left renal artery. Taking care not to disturb the left kidney, the aorta was occluded with a Potts clamp placed between the left and right renal arteries. This diverted the entire output of the pump through the left

kidney. By adjusting the pump renal blood flow was maintained at an average value of 75 ml/min (range 50 to 150 ml/min) which produced a mean arterial perfusion pressure of approximately 100 mm Hg. This provided a blood flow of approximately 2-3 ml/gm of kidney tissue. After traversing the renal vascular bed, the pumped blood returned to the body pool via the renal vein.

Renal arterial and systemic arterial pressures were measured by a needle inserted in the perfusion tubing just proximal to the glass cannula, and by needling the proximal aorta, respectively. Renal venous pressure was obtained by needling the renal vein. These pressures were recorded on a direct writing oscillograph recorder connected to Statham pressure transducers. Renal vascular resistances were calculated by dividing the pressure gradient from renal artery to renal vein by the blood flow per 100 gm kidney weight. The resistance units were expressed as mm Hg/ml/min/100 gm kidney weight. Statistical analysis was done using the "Student T test."

Endotoxin. The dose of Salmonella typhosa 0901 (Bacto Lipopolysaccharide, Difco Laboratories)--whether irradiated or unirradiated--administered was 0.6 mg/kg. This dosage is always lethal for the dog. It was injected from a calibrated syringe by hand in one bolus directly into either the perfusion system just proximal to the renal artery or into a systemic vein. Renal and systemic pressures were recorded for 30 minutes following an injection of endotoxin. Base line pressures were recorded for at least 10 minutes previous to each injection. For control purposes, five animals were followed over a 30-minute period without administration of endotoxin, and 15 dogs were given nonirradiated endotoxin directly into the perfused renal artery. Endotoxin radiated at 10.40 Mc/sec and at 13.34 Mc/sec was administered to groups of 13 and 14 dogs, respectively. Endotoxin was radiated in vitro in 2.5 ml batches (enough for one dog) or in 5 ml batches (enough for two dogs). The last eight experiments in the nonirradiated 10.40 Mc/sec and 13.34 Mc/sec irradiated groups represent experiments done six months after the initial experiments. In the latter experiments completely different instrumentation was used to radiate the endotoxin. Since the results were identical with the ones obtained six months earlier, the results were combined in the figures and tables accompanying this report.

Electronic Apparatus. A Hewlett-Packard VHF Signal generator (Model 608D) was used as a source of energy for radiating endotoxin samples. From this source the signal was amplified through a cascade

of low power voltage amplifiers (one IFI* Model 530, and two IFI* Model 500A) and a wideband power amplifier (IFI* Model 400). The amplified signal was then passed through directional couplers (two Bendix Micromatch Model 252N1) arranged back to back. This arrangement of couplers permitted the measurement of incident and reflected power to the exposure chamber (Fig. 1). These measurements were used to determine the voltage standing wave ratio (VSWR). The VSWR in all exposures of endotoxin was less than 1.08.

The exposure chamber (a tunable circuit) consisted of a coil which surrounded a glass water jacket (Fig. 2). The water jacket held the test tube containing the endotoxin sample in the R.F. energy field. Changes in the temperature of the coolant were monitored by thermistor probes (Yellow Springs Instrument Company) in the inlets and outlets of both the energized and non-energized exposure chambers. To minimize temperature increases in the sample of endotoxin during radiation, high coolant flow rates were used (10 liters per minute). Coolant temperatures were stabilized at 12.5° C. No differences in the temperatures of the affluent and effluent coolant were observed during the exposures.

The power delivered to the energized exposure chamber was 15.0 watts; .4 watt was reflected. Thus, the apparent power delivered was 14.6 watts. All power measurements were made from the directional couplers. For consistency in experimental technique and to insure identical exposure conditions, all power measurements were replicated during each radiation experiment.

III. RESULTS

Control Data. No significant changes were observed in renal artery, renal vein, or systemic arterial pressure of five dogs** (10) in which pressures were recorded continuously for a 30-minute period for control data purposes (Table 1).

Nonirradiated Endotoxin.

Renal Arterial Injection. Injection of S. typhosa endotoxin into the perfused renal artery produced a characteristic diphasic

*Instruments for Industry.

**These data on the control animals and animals receiving nonirradiated endotoxin have been previously reported (USAMRL Report No. 551, 1962) (10).

pressor response illustrated by the photograph of a representative record shown in Figure 3. First, there was an immediate transient mild rise in renal pressure which rapidly returned to the pre-injection level. Subsequently, a second delayed and greater increase in renal pressure developed progressively, reaching a maximum in 5-10 minutes after which it returned toward, or to, the normal level where it remained for the duration of the 30-minute recording.

There was little, if any, variability from one animal to another in the characteristic of the initial transient pressor response to endotoxin. However, there was considerable variability in the magnitude and duration of the second delayed pressor response. This is shown by the family of curves covering each of the 15 experiments shown in Figure 4 and the data in Table 2. However, the response-pattern, a rise in pressure with subsequent return to or toward the pre-injection level, is the factor which is common to each experiment.

Systemic Injection. The renal vascular response to endotoxin injected systemically was also diphasic (10). The first pressor response was, of course, slightly delayed and was less pronounced. The second delayed pressor response was similar to that following intrarenal artery injection; the same variability in the magnitude of response and, except in two instances, the same response-pattern. The family of curves covering each of eight such experiments is shown in Figure 5. The exceptions in response-pattern are marked with X on the right. Note that in these exceptions the pressor response was sustained; there was no return toward the pre-injection level during the 30-minute run.

Irradiated Endotoxin.

10.40 Mc/sec. Injection of endotoxin previously radiated with 10.40 Mc/sec radio-frequency energy also produced the characteristic diphasic response but the response-pattern differed in certain respects from that produced by nonirradiated endotoxin. A representative record is reproduced in Figure 6, the family of curves covering each of 13 experiments is shown in Figure 7, and the data covering each experiment are presented in Table 3.

The first transient response was uniformly greater in magnitude following the 10.40 Mc/sec radiated than following the nonirradiated endotoxin. This is readily evident by comparing the height of the first response in Figures 5 and 6, and by the data presented in Tables 2 and 3.

There was also a definite change in the second delayed pressor response-pattern subsequent to radiation of the endotoxin by 10.40 Mc/sec. First, there was less variation in the magnitude of the increase in pressure and, secondly, and more important, is that uniformly, except in one instance, the maximal height in pressure attained was sustained for the duration of the 30-minute recording; there was no return to or toward the pre-injection levels as occurred after the injection of unirradiated endotoxin.

This difference in the response-pattern between 10.40 Mc/sec irradiated and nonirradiated endotoxin is readily evident by comparing the tracing in Figures 3 and 6 and the families of curves in Figures 5 and 7. This difference is particularly well emphasized and characterized by the average data for the two different groups of experiments shown in Figure 8.

13.34 Mc/sec. Injection of endotoxin previously radiated with 13.34 Mc/sec enhanced the first pressor response but the second pressor response did not differ noticeably from that produced by unirradiated endotoxin. This is evidenced by the family of curves covering the individual experiments shown in Figure 9, by the data tabulated in Table 4, and the average data shown in Figure 10.

IV. CONCLUSIONS

The foregoing data clearly demonstrate that radiating the endotoxin material in vitro with 10.40 Mc/sec, altered its pharmacodynamic properties as evidenced by a characteristic and predictable change in the pressor response-pattern elicited in the renal vascular bed. The radiofrequency range of 10.40 Mc/sec was in some way specific because radiating with a frequency of 13.34 Mc/sec did not cause as great a change in pharmacodynamic properties of the endotoxin material. The nature of the change (physical, chemical?) in this material induced by 10.40 Mc/sec radiation remains problematical. However, to be emphasized is the fact that the pharmacodynamic properties of this material was enhanced by radiation rather than being attenuated as reported in other investigations (2, 3).

The initial transient pressor response is reasonably certain to be a direct effect of the endotoxin on the renal vascular musculature. First, this pressor response occurred before the endotoxin had time to return to the renal bed after circulating through the body blood pool when the endotoxin was introduced directly into the renal artery. This

response appeared later and the pressor effect was not as great when the endotoxin was administered systemically. Secondly, the initial pressor response was not altered, whether given directly or systemically, by the previous administration of phentolamine or reserpine (10).

The second more pronounced pressor response has been previously demonstrated to be due to the action of catecholamines released into the blood stream when the endotoxin reaches the systemic circulation (11). It is generally believed that endotoxin, in addition to directly exciting vascular musculature, also sensitizes this musculature to catecholamines (12).

V. RECOMMENDATIONS

1. It is recommended that the nature of the change (physical, chemical?) induced in the S. typhosa material by radiation with 10.40 Mc/sec be investigated.

2. It is recommended that it be determined whether radiation of this material alters its lethal characteristics by using an appropriately designed experimental series with small animals such as cockerels or mice.

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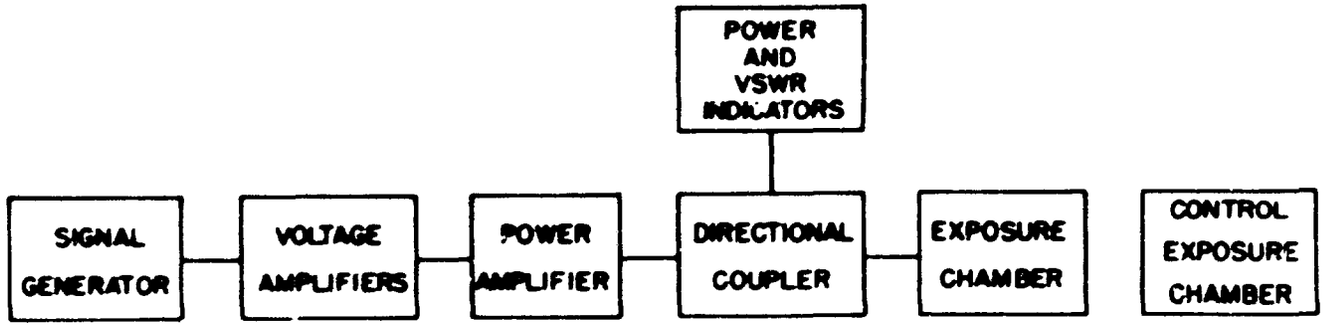

Fig. 1. Diagrammatic scheme of the electronic apparatus.

EXPOSURE ARRANGEMENT FOR SOLUTIONS

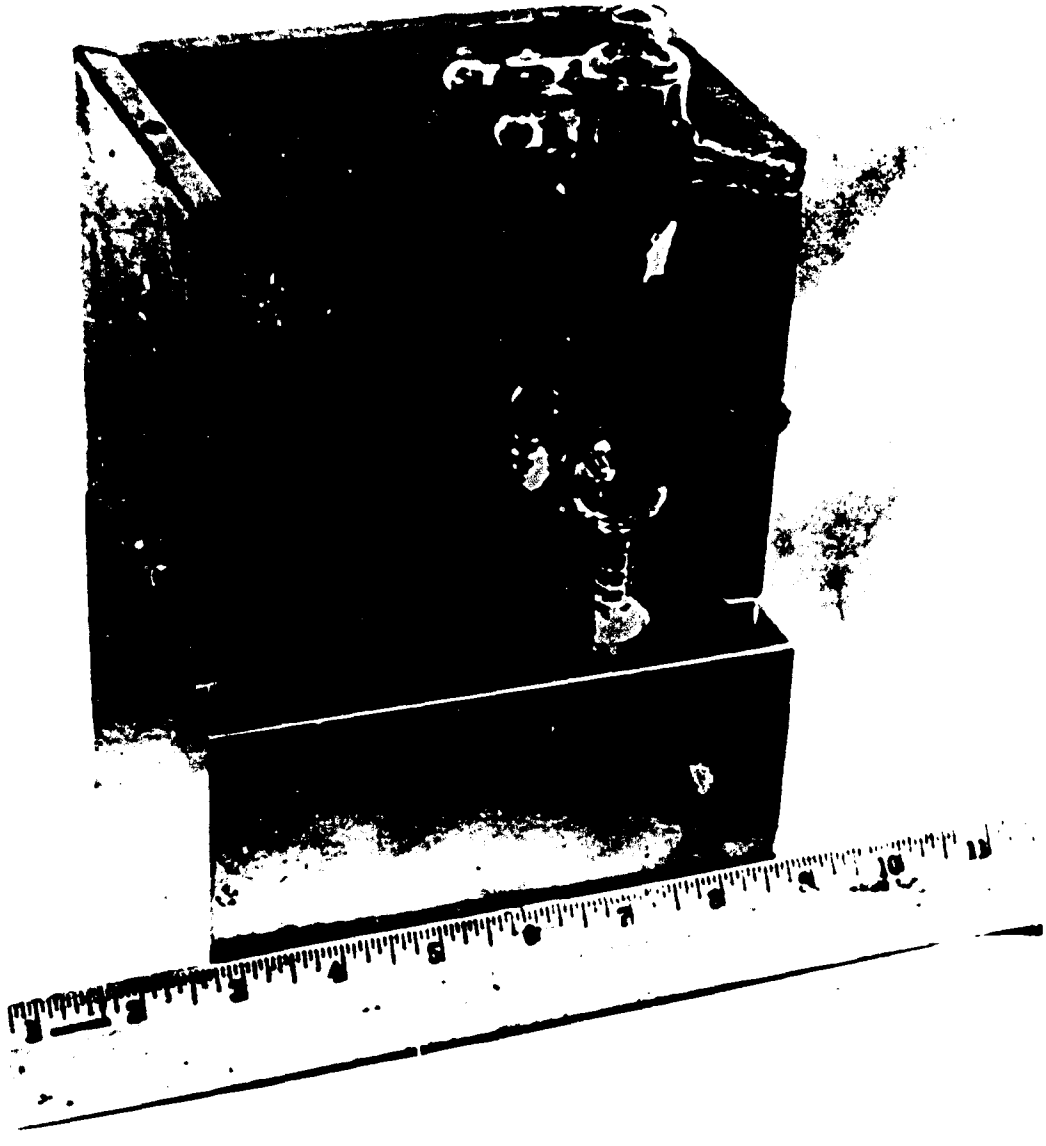

Fig. 2. The exposure chamber (a tunable circuit) consisting of a coil which surrounded a glass water jacket that was used to radiate the endotoxin.

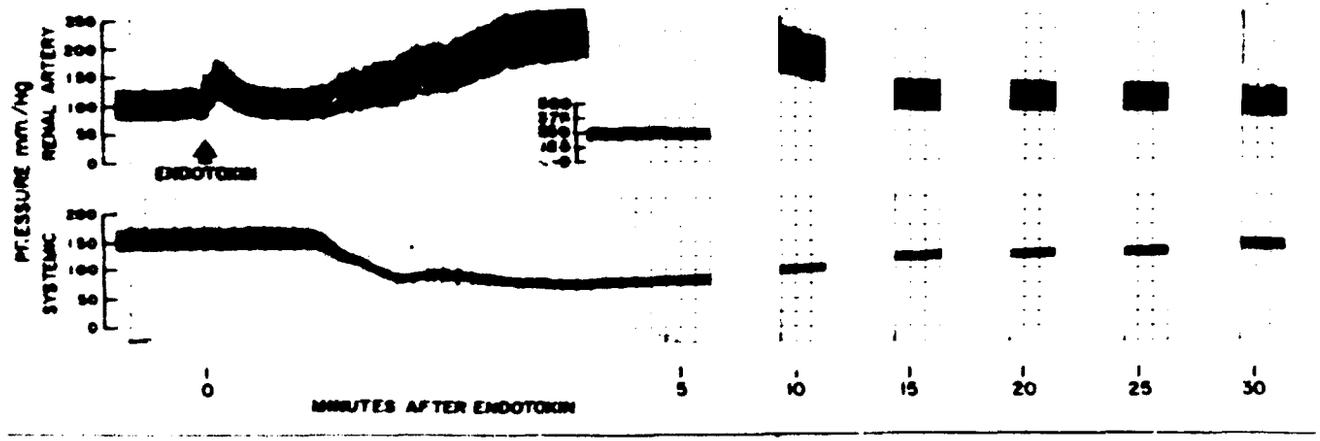

Fig. 3 A representative tracing of an experiment in which the nonirradiated endotoxin was administered locally into the perfused renal artery. The initial transient vasoconstriction followed by the secondary sustained vasoconstriction can be seen.

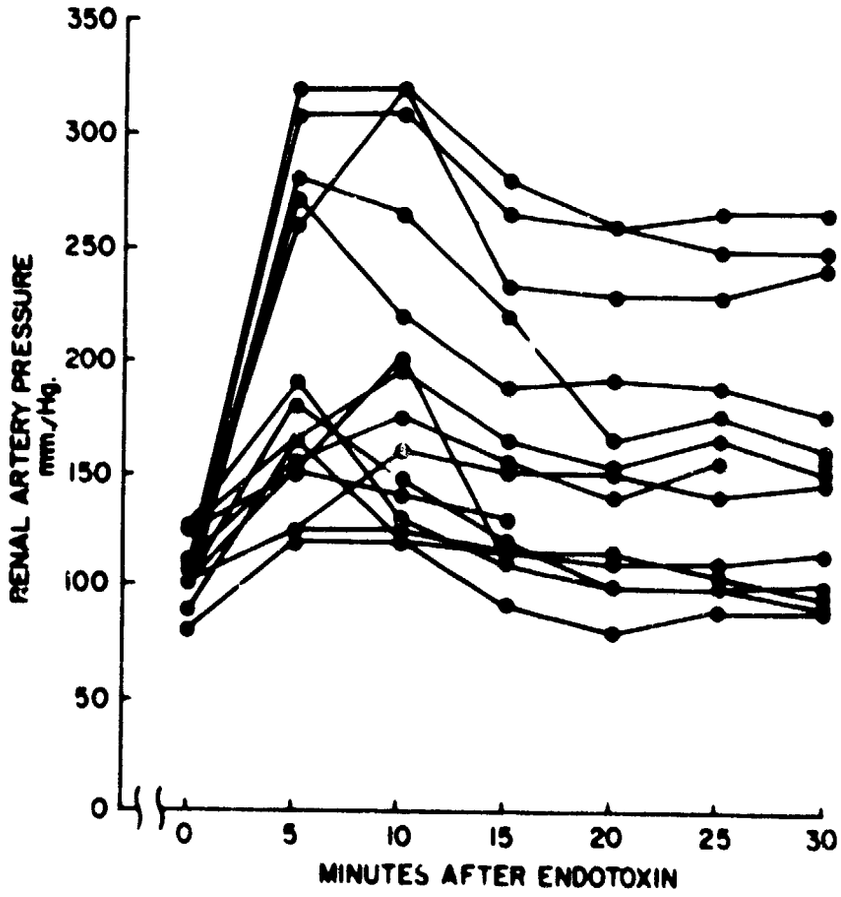

Fig. 4. The renal artery pressure of the 15 dogs in which non-irradiated endotoxin was injected into the perfused renal artery.

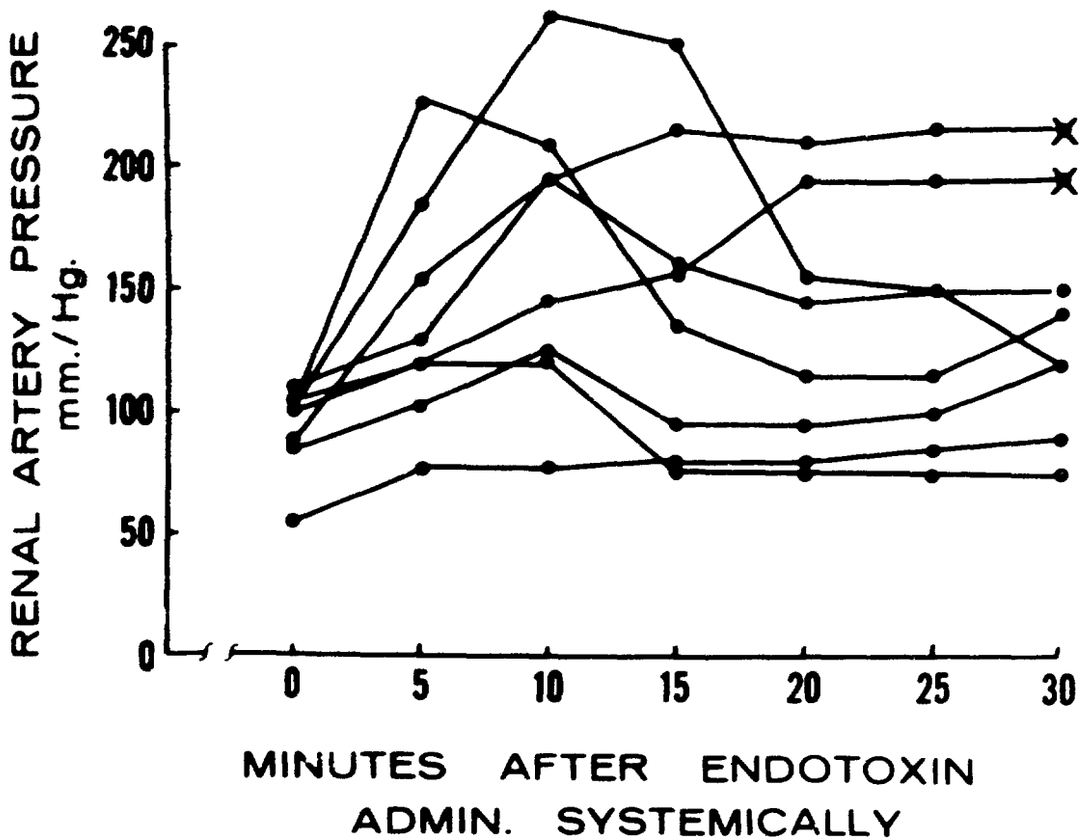

Fig. 5. The renal artery pressure of the eight dogs in which nonirradiated endotoxin was injected systemically.

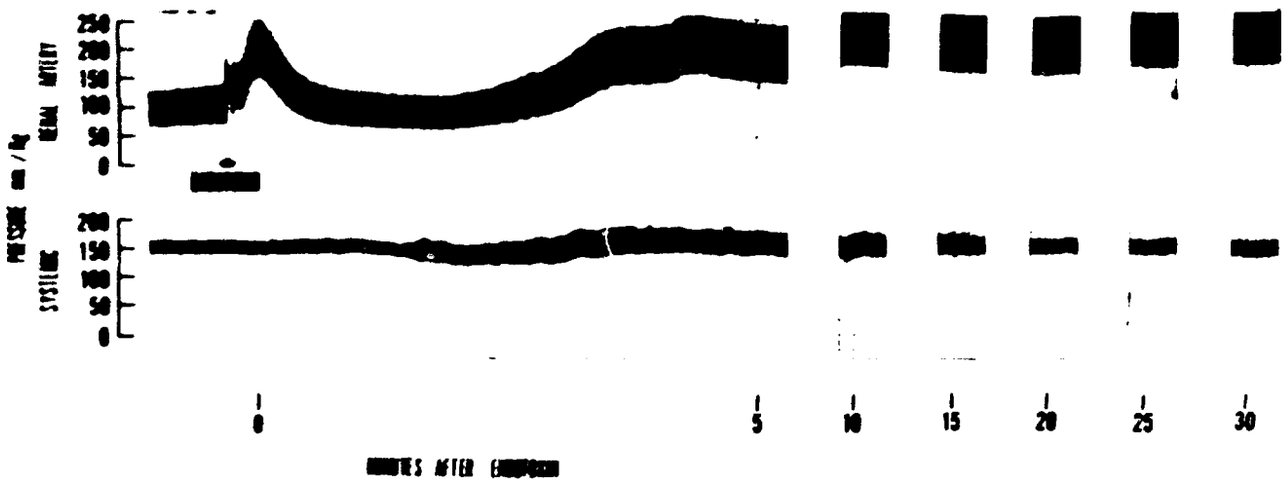

Fig. 6. A representative tracing of an experiment in which 10.40 Mc/sec irradiated endotoxin was injected into the perfused renal artery.

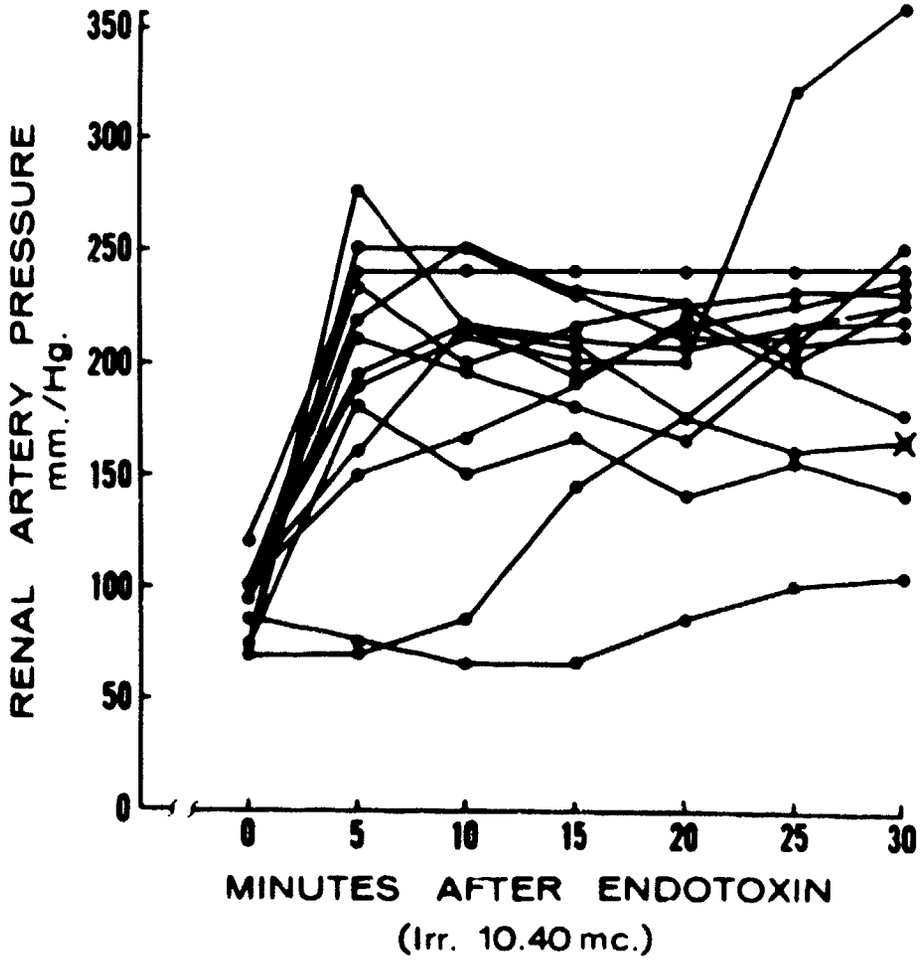

Fig. 7. The renal artery pressure of the 13 dogs in which 10.40 Mc/sec irradiated endotoxin was injected into the perfused renal artery.

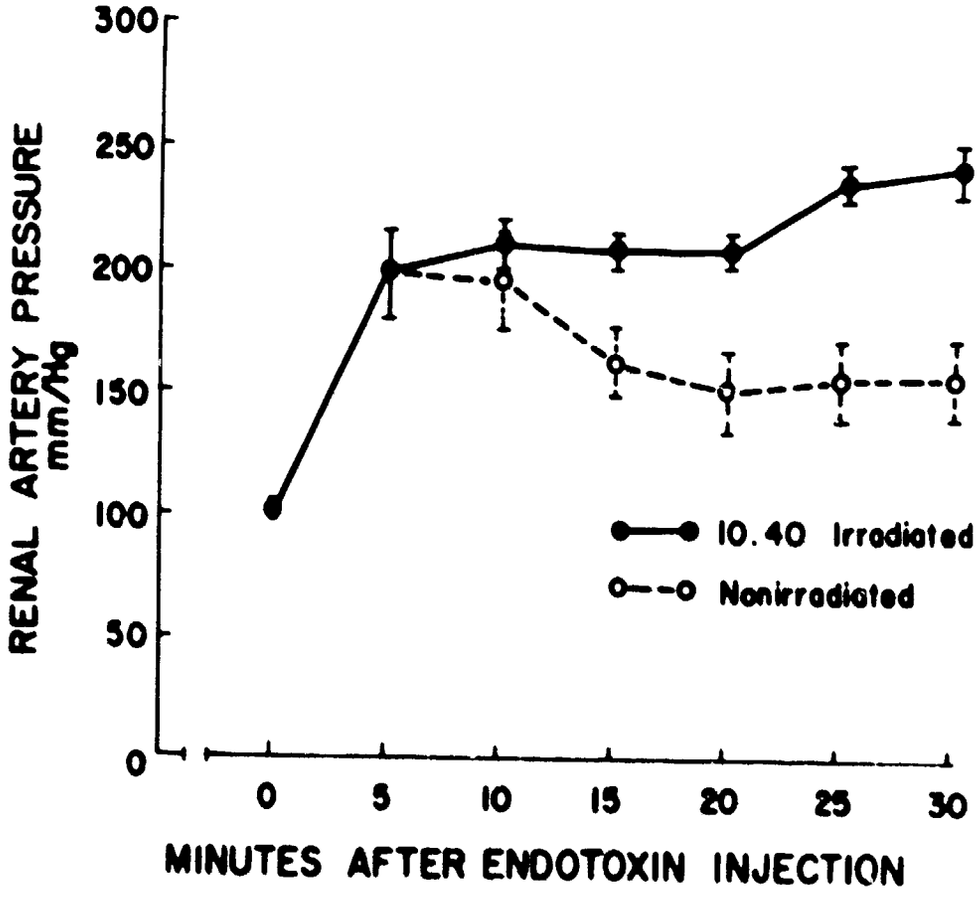

Fig. 8. The mean renal artery pressures of the 10.40 Mc/sec irradiated group and the nonirradiated group.

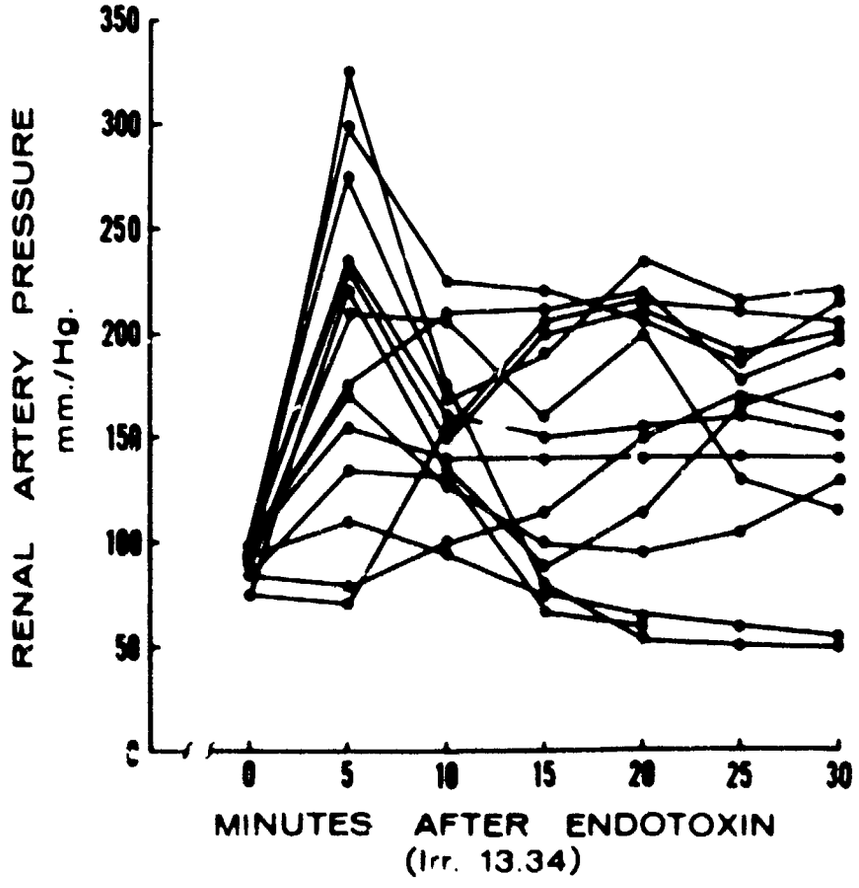

Fig. 9. The renal artery pressures of the 14 dogs in which 13.34 Mc/sec irradiated endotoxin was injected into the perfused renal artery.

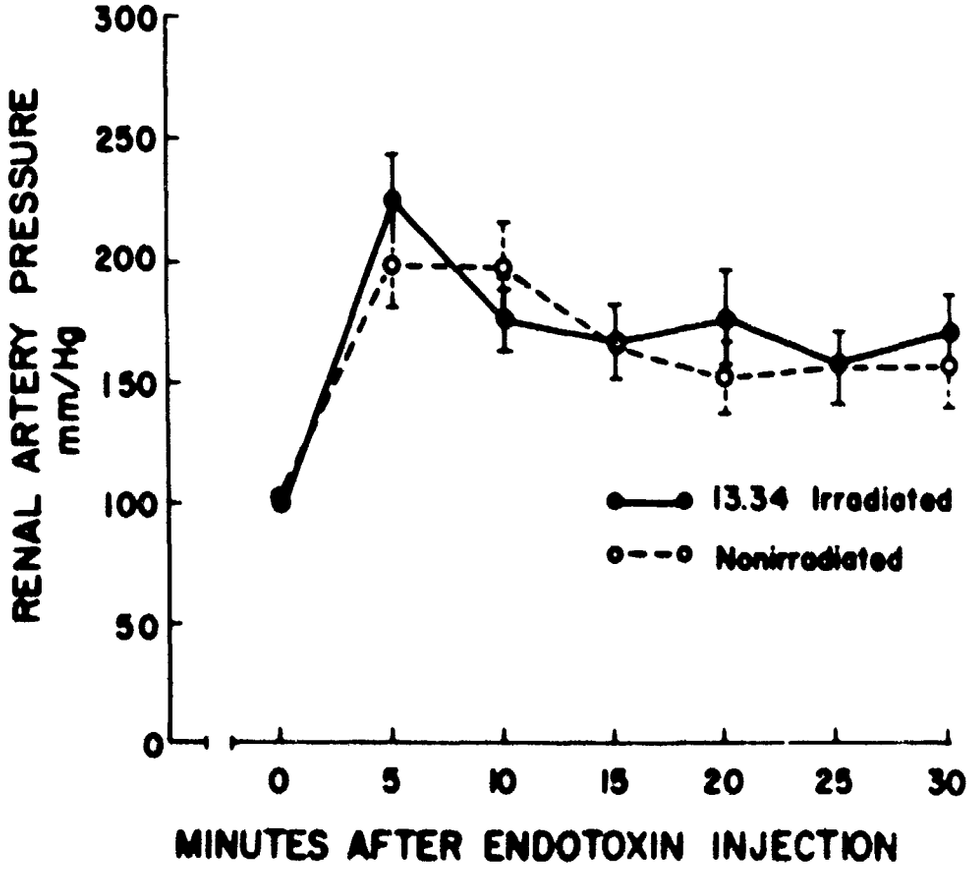

Fig. 10. The mean renal artery pressures of the 13.34 Mc/sec irradiated group and the nonirradiated group.

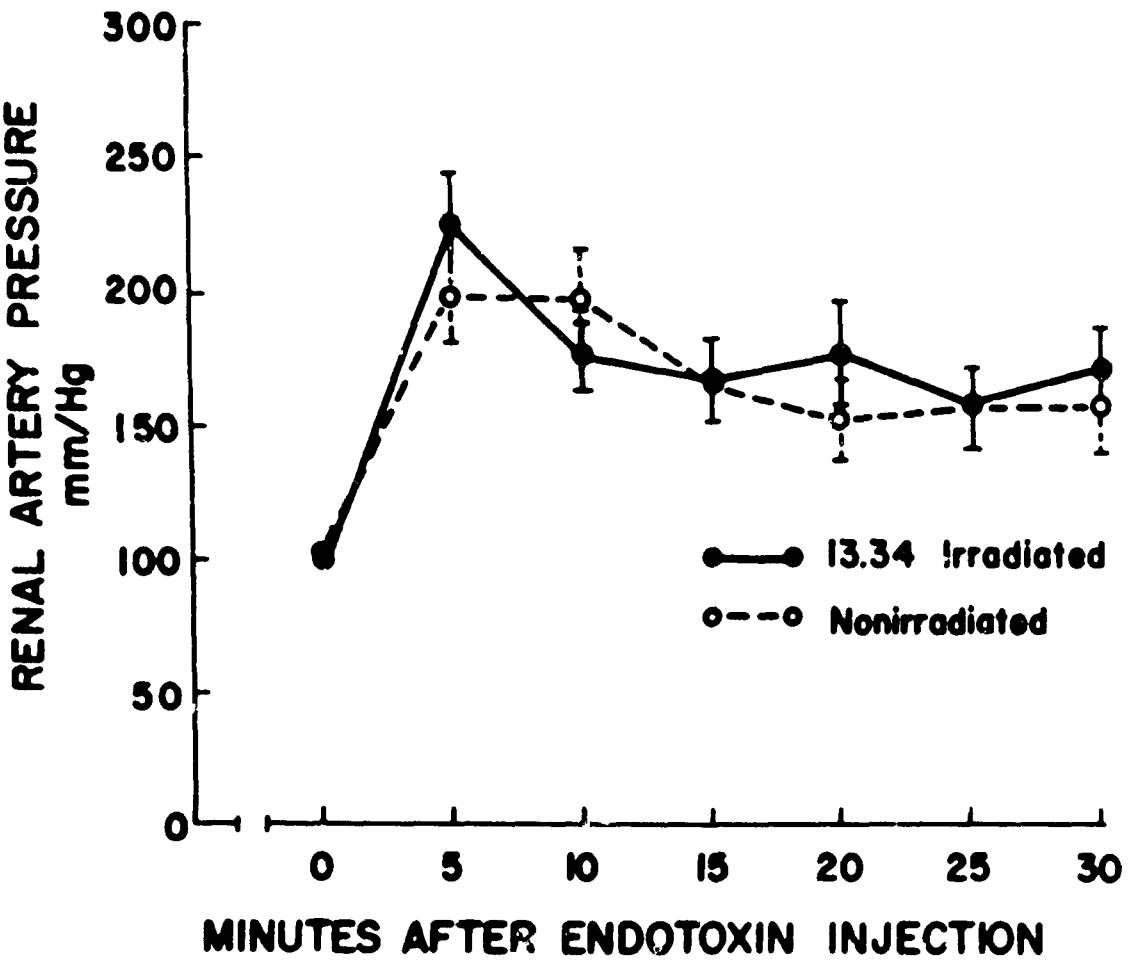

Fig. 10. The mean renal artery pressures of the 13.34 Mc/sec irradiated group and the nonirradiated group.

TABLE 1. Renal artery pressures of the control animals.

		Delayed Pressor Response					
Time (min)	0	5	10	15	20	25	30
Exp. No.							
1	100	100	100	104	108	108	104
2	90	90	92	90	82	90	95
3	90	92	90	95	87	90	95
4	107	105	105	105	100	95	95
5	97	97	98	100	97	99	97
Mean	97	97	97	99	95	96	97
S.E.	±3.3	±2.7	±2.7	±2.7	±4.6	±3.4	± 2

TABLE 2. Renal artery pressures of the dogs in which nonirradiated endotoxin was injected directly into the perfused renal artery. Average kidney weight was 33 gm and average perfusion flow was 70 ml/min.

		First Pressor Response		Second Pressor Response					
Time (min)	0	Peak	1	5	10	15	20	25	30
Exp. No.									
1	100	130	72	272	220	188	192	188	176
2	88	148	100	164	120	92	80	88	88
3	104	200	144	320	320	232	228	228	240
4	80	100	90	120	120	116	116	104	96
5	104	140	110	260	320	280	260	248	248
6	100	125	100	180	148	120	100	100	100
7	100	150	104	152	200	112	-	-	-
8	124	152	120	150	140	130	60	-	-
9	100	180	150	165	195	165	153	165	150
10	100	220	135	280	266	220	165	175	160
11	100	150	104	125	160	150	150	140	145
12	100	150	105	125	125	115	110	110	115
13	105	165	125	308	308	266	257	266	266
14	125	175	150	190	130	110	100	100	90
15	110	225	150	155	175	155	140	155	155
Mean	103	161	117	198	196	163	151	155	156
S.E.	± 2.9	± 9.5	± 6	± 18	± 21	± 15	± 17	± 17	± 17
			Calculated resistance mm Hg/ml/min/100 gm kidney weight						
Mean	49.5	59.4	48.8	94.1	93.4	77.6	75.5	75.	73.9
S.E.	± 1.7	± 6.6	± 4.0	± 5.6	± 4.3	± 3.0	± 3.3	± 3.3	± 3.0

TABLE 3. Renal artery pressures of the dogs in which 10.40 Mc/sec irradiated endotoxin was injected directly into the renal artery. Average kidney weight was 42 gm and average perfusion flow was 80 ml/min.

		First Pressor Response		Second Pressor Response					
Time (min)	0	Peak	1	5	10	15	20	25	30
Exp. No.									
1	85	123	75	75	65	65	85	100	112
2	70	170	90	70	85	145	175	215	225
3	70	190	135	275	215	210	175	160	165
4	70	185	120	180	150	165	140	155	140
5	75	170	105	250	250	235	225	200	225
6	100	215	150	150	165	190	220	195	175
7	120	225	175	240	240	240	240	240	240
8	100	200	180	235	200	215	225	230	230
9	100	225	155	220	250	230	210	210	250
10	100	175	150	160	215	195	215	225	235
11	100	200	150	190	212	200	200	322	364
12	103	160	120	195	217	210	205	215	217
13	95	225	175	210	196	180	165	207	210
Mean	91	190	137	180	189	191	191	199	214
S. E.	± 4.0	± 8.3	± 8.6	± 18	± 16	± 13	± 12	± 14	± 18
			Calculated resistance mm Hg/ml/min/100 gm kidney weight						
Mean	50	104	75	105	105	107	107	116	112
S. E.	± 5	± 11	± 10	± 11	± 11	± 10	± 10	± 9	± 9

TABLE 4. Renal artery pressures of the dogs in which 13.34 Mc/sec irradiated endotoxin was injected directly into the renal artery. Average kidney weight was 41 gm and average perfusion flow was 85 ml/min.

	First Pressor Response		Second Pressor Response						
Time (min)	0	Peak	1	5	10	15	20	25	30
Exp. No.									
1	85	140	90	135	130	65	60	-	-
2	75	155	80	70	155	205	215	210	205
3	85	187	177	80	100	115	150	170	160
4	95	115	90	110	95	75	65	60	55
5	75	220	150	200	130	90	115	165	175
6	90	180	95	325	175	80	55	50	50
7	100	175	180	230	150	200	215	190	200
8	100	225	188	175	210	210	215	175	200
9	100	150	140	172	130	100	95	105	130
10	102	215	125	155	140	137	140	140	140
11	95	240	225	225	210	160	200	130	115
12	95	200	125	235	160	100	100	110	150
13	95	200	125	300	225	220	210	185	215
14	100	200	140	275	157	192	235	215	215
Mean	92	186	138	192	155	139	148	147	155
S.E.	± 4.0	± 10	± 11	± 21	± 11	± 15	± 18	± 18	± 16
	Calculated resistance mm Hg/ml/min/100 gm kidney weight								
Mean	44.4	90	67	90	74	66	70	71	72
S.E.	± 1.8	± 8	± 10	± 9	± 5	± 7	± 8	± 6	± 6