grade 2.9) and there was transitory loss of weight. In contrast, the group that received both encephalitogenic emulsion and cobalt implant had no loss of weight, signs of EAE were milder (average severity only 1.6) and 7 rats had no signs at all. The difference in EAE was confirmed histologically. This partial suppression of EAE was probably due to a non-specific stress effect of the cobalt implant(5). An effect on the hypothalamus of the large necrotic focus around the frontal lobe implant, with consequent pituitary-adrenal stimulation, cannot be ruled out.

Summary. Rats with experimental allergic encephalomyelitis (EAE) had reduced threshold to Metrazol-induced convulsions. The threshold of rats with cobalt implants was even lower. Rats with both EAE and cobalt implants had the same threshold as rats with cobalt implants only. The severity of EAE was reduced in this group, probably due to non-specific stress effects of the cobalt implants.

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Effect of *Pseudomonas pseudomallei* Endotoxin on Response to Cholinergic Stimuli in the Rabbit.* (28526)

G. J. HILDEBRAND, M. REDFEARN AND Y. SEYS (Introduced by A. P. Krueger)
(With the technical assistance of James Ng)
Naval Biological Laboratory, School of Public Health, University of California, Berkeley

Increased peristalsis, sustained rise in right ventricular pressure and fall in arterial pressure to shock level were among the effects produced by intravenous injection of *Pseudomonas pseudomallei* endotoxin in rabbits. These responses were similar to those produced in rabbits and other animals by various bacterial endotoxins(2,3,4). Since it appeared that some of these effects might result from hyperactivity of cholinergic mechanisms that became sensitized to cholinergic stimuli by endotoxin, we investigated the possibility of an enhanced response to cholinergic stimuli in rabbits injected with *P. pseudomallei* endotoxin.

Materials and methods. Female New Zealand white rabbits, weighing from 3.1 to 5.9 kg were used. The animals were anesthetized by intravenous (i.v.) injection of sodium phenobarbital (100 mg/kg) and sodium pentobarbital (15 mg/kg).

Endotoxin was isolated from cultures of *P. pseudomallei* strain 118-6 (rough), that were grown in a chemically defined medium(6) containing 0.1 M gluconolactone, 0.1 M KH_2PO_4, 0.0045 M $MgSO_4 \cdot 7H_2O$, 0.000023 M $FeSO_4 \cdot H_2O$. The medium was adjusted to pH 6.4 with NH_4OH. The organisms were killed with formalin at a final concentration of 1% and extracted with phenol by the method of Westphal(5). The phenol extract containing endotoxin was fractionated by precipitation with acetone and differential centrifugation. The endotoxin isolated contained less than 2% nitrogen and was devoid of both protein and nucleotide residues. The

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i.v. LD_{50} of the endotoxin in rabbits, as estimated by the mortality grid method of Goldberg(7), was 12.5 μg/kg. The true value was contained in the interval 7.5 to 21 μg/kg with a probability of 0.95.

Endotoxin was injected into the jugular vein in doses ranging from 0.5 to 50 μg/kg, corresponding to approximately LD_5 (1 μg/kg) and LD_{95} (55 μg/kg) respectively.

Acetylcholine (AC) was used for chemical stimulation of cholinergic mechanisms. AC in constant concentration was infused into the left jugular vein at constant rates for variable lengths of time, to achieve reproducibility between individual doses. The infusions were usually made at 5-minute intervals. For direct or reflex stimulation of cholinergic structures, we stimulated the peripheral end of either vagus nerve or the central end of either depressor nerve. Parameters were chosen to produce a slowing of the heart rate by approximately 30% (volts: 2.4 to 5, duration: 2 milliseconds, frequency: 20 and 100/second).

Criteria of the effectiveness of the cholinergic stimuli were systolic pressure in the right ventricle, diastolic arterial pressure and cardiac rate and rhythm. The right ventricle was intubated through the right jugular vein with a polyethylene tube (No. 100). Blood pressure, in both the pulmonary artery and the left atrium, and intratracheal pressure were recorded in open thorax preparations under artificial positive-pressure respiration. The femoral artery was used to record the arterial pressure in the major circulation. All pressures were transmitted to Statham transducers and recorded with a Honeywell visicorder. Cardiac rate and rhythm were determined from the electrocardiogram (ECG).

Results. Effect of AC on systolic right ventricular pressure (RVP) in animals injected with endotoxin. Pre-endotoxin injections of AC in doses of 0.12 to 0.32 μg/kg resulted in a fall in RVP of 4 to 22 mm H_2O in 17 of 32 animals and in a rise of 5 to 20 mm in 8 animals. These responses lasted from 0.5 to 2.5 minutes. There was no change in the RVP of the remaining 7 animals. Dose-response relationships were ill defined. No cumulative effects were observed with these small doses even when they were repeated at 5-minute intervals.

Fig. 1 is representative of the response in RVP to AC injected before and after administration of endotoxin. After injection of endotoxin, a latent period of 12 to 50 minutes was observed, during which there was no change in the response to AC. After this latent period, 29 of 32 animals that had received endotoxin responded to AC injections with a transient rise in RVP, which increased in magnitude and duration with each subsequent injection of AC. Maximal responses ranging from 28 to 180 mm H_2O and lasting from 2 to 6 minutes were attained 16 to 62 minutes after injection of endotoxin. Subsequently, in 17 of these animals, the rise in RVP decreased in magnitude and duration with the larger doses of AC and tended to disappear with the smaller doses. Some of these animals showed a cyclical increase and decrease in RVP (Fig. 1). In the other 12 rabbits, the first or second injection of AC after the latent period elicited a sustained rise in RVP (Fig. 2).

Five animals received a second injection of endotoxin when the response to AC had subsided. After reinjection of endotoxin, successive injections of AC again produced a progressive increase in RVP, although the maximal response was not, as a rule, as great as that observed after the initial injection of the endotoxin. But there was no latent period or a considerably shorter one than that observed after the first injection of endotoxin.

A similar response to AC injections was obtained when the blood pressure in the pulmonary artery, instead of that in the right ventricle, was determined. This showed that the results were not referable to intubation of the right ventricle. Blood pressure in the left atrium was not elevated prior to or during the rise in pulmonary arterial pressure. Intratracheal pressure did not increase significantly during the pressor response to AC in the pulmonary artery.

With a hundred-fold increase in the dose of endotoxin, there was a significant decrease in duration of the latent period, and a significant increase in duration and magnitude

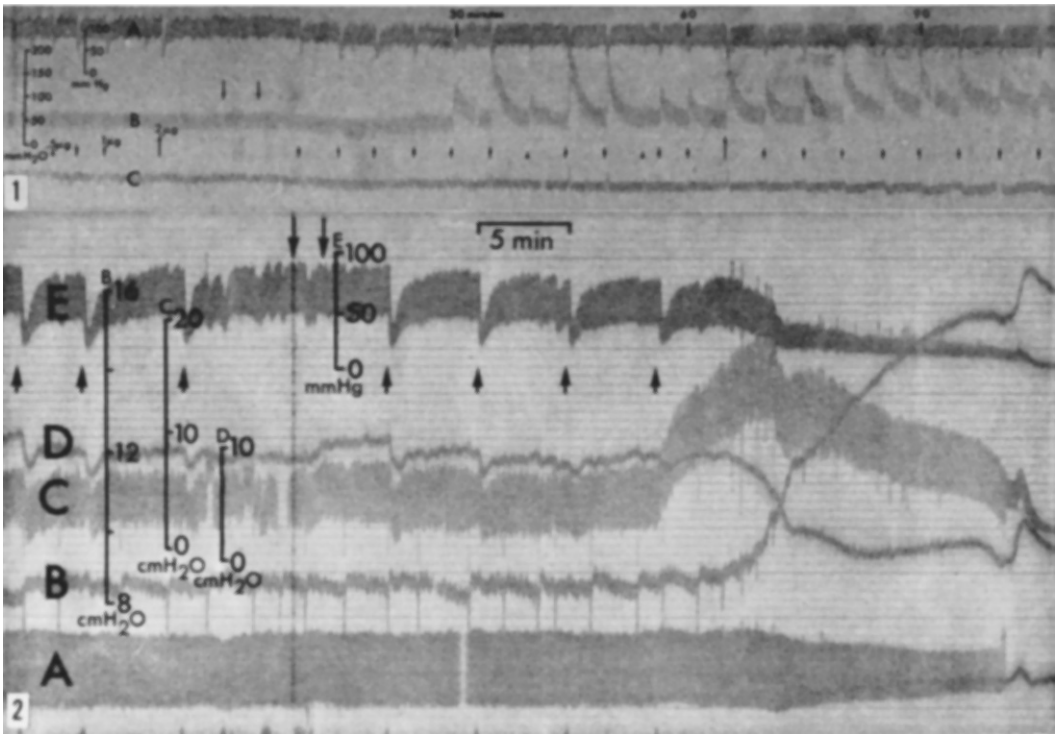

FIG. 1. Effect of *Pseudomonas pseudomallei* endotoxin on response in arterial (A), right ventricular (B), and central venous pressure (C) to intravenous injection of acetylcholine. Two arrows ↓↓ indicate beginning and end, respectively, of intravenous injection of endotoxin (0.5 μg/kg/min). Injection of acetylcholine in doses of 0.5, 1.0 and 2.0 μg/animal is indicated by arrows (↑) graduated in length respectively. Time is in minutes after injection of endotoxin.

FIG. 2. Lethal effect of intravenous injection of acetylcholine in a rabbit treated with *Pseudomonas pseudomallei* endotoxin. Arrows ↓↓ indicate beginning and end, respectively, of injection of 50 μg endotoxin/kg. Arrows ↑ indicate intravenous injection of 0.3 μg acetylcholine/kg. A—thoracic plethysmogram, B—central venous pressure, C—right ventricular pressure, D—portal pressure, E—arterial pressure. Time in minutes after injection of endotoxin.

of the maximal response in RVP (Table I). For a given dose of endotoxin, the duration of the latent period varied inversely with the dose of AC. However, even 500 μg endotoxin/kg and 0.7 μg AC/kg failed to reduce the latent period to less than 12 minutes.

Effect of AC on arterial diastolic pressure in animals injected with endotoxin. Usually the fall in arterial diastolic pressure, produced by AC, increased in duration rather than in magnitude after injection of endotoxin. The enhanced vasodilating action of AC may have been obscured by the arterial hypotension produced by injection of endo-

TABLE I. Response in Right Ventricular Systolic Pressure to Acetylcholine Injected Intravenously into Rabbits Treated with *Pseudomonas pseudomallei* Endotoxin.

			Response in right ventricular systolic pressure*					
			Before injection of endotoxin		After injection of endotoxin			
	Dose (μg/kg) of				Maximal response			Duration of latent period (min)
No. of rabbits	Endotoxin	Acetylcholine	Magnitude (mm H_2O)	Duration (min)	Magnitude (mm H_2O)	Duration (min)	Time (min)	
10	50	.37 ±.03	−10.3 ± 7	.83 ±.94	+129 ± 33	4.5 ± 1.6	32.4 ± 5.7	21 ± 3
6	.5	.36 ±.08	− 5 ±11	.3 ±.5	+ 75 ± 37	3.5 ± .9	43 ±11	33 ± 10

* + rise in pressure; − fall in pressure.

toxin. Such hypotension is commonly attributed to vasodilatation and pooling of blood in some peripheral vascular areas of the major circulation(2,14). However, fall in arterial pressure may be intensified by vasoconstriction of the pulmonary vessels, resulting in reduced filling and reduced stroke volume of the left ventricle. Such a mechanism was indicated in 4 animals in which arterial pressure fell to shock level after the pronounced rise in RVP evoked by AC (Fig. 2). Low arterial pressure and increased RVP persisted for 5 to 20 minutes when right ventricular failure occurred, as indicated by a sudden rise in central venous pressure (Fig. 2). However, some animals responded to injection of AC after administration of endotoxin with a greater fall in arterial pressure, although concomitant vasoconstriction in the pulmonary vascular bed and arterial hypotension were not observed. No valid conclusions could be drawn from these experiments as to whether endotoxin enhanced the response of cholinergic vasodilator mechanisms in the major circulation to injection of AC.

Effect of AC on central venous pressure and portal pressure after injection of endotoxin. AC produced only irregular and minimal changes in both central venous pressure (Fig. 1) and in portal pressure (Fig. 2), either before or after injection of endotoxin.

Effect of AC on cardiac rate and rhythm of animals injected with endotoxin. The low doses of AC employed in these experiments did not produce changes in cardiac rate either before or after injection of endotoxin. Ventricular extrasystoles were observed only after injection of endotoxin and whenever AC produced a pronounced rise in RVP. Moreover, they disappeared when the polyethylene tube was removed from the right ventricle and were not observed when the blood pressure in the pulmonary artery was recorded. These arrhythmias probably resulted, therefore, from mechanical irritation of the ventricular wall by the polyethylene tube inserted into the right ventricle. It could not be determined from our experiments whether mechanisms such as hyperexcitability of the cardiac muscle, rotation of the axis of the heart, or a more vigorous action of the right ventricle during the rise in RVP caused these mechanically induced arrhythmias to appear only after administration of the endotoxin.

Effect of stimulation of parasympathetic nerves. Stimulation of the vagus or depressor nerves produced a slight fall in arterial pressure and a slight rise or fall in RVP similar to that produced by AC. Injection of endotoxin did not alter these responses to parasympathetic stimulation, although the animals responded to AC with a pronounced rise in RVP.

Effect of AC on blood pressure in rats and guinea pigs after injection of endotoxin. Post-endotoxin injections of AC failed to produce changes in RVP and carotid blood pressure of 4 rats and 4 guinea pigs, although doses of 500 and 1000 μg endotoxin/kg and 2 to 6 μg AC/kg were injected.

Discussion. Intravenous injection of *P. pseudomallei* endotoxin into rabbits increased the response of pulmonary cholinergic vasoconstrictor mechanisms to i.v. injection of AC. However, there was no clear evidence that endotoxin intensified the response of the cholinergic vasodilator mechanisms in the major circulation to AC. Other investigators have found an abrupt fall in arterial pressure resulting in death, after injection of large doses of AC (20 to 50 μg) into rabbits that had received *S. typhi abdominalis* endotoxin (8). We observed that smaller doses of AC (0.5 to 2 μg) produced a similar response in rabbits injected with *P. pseudomallei* endotoxin. This was the effect of extreme vasoconstriction in the pulmonary circulation, with decreased stroke volume of the left ventricle and acute right ventricular failure, and not of vasodilatation in the major circulation. Cardiac rate and rhythm, portal pressure, central venous pressure and resistance in the respiratory passages were not significantly affected by injection of AC either before or after injection of endotoxin. Endotoxin also did not alter the response to stimulation of parasympathetic nerves. It is not clear why endotoxin did not enhance the effects of AC liberated in the cholinergic structures upon stimulation of their parasympathetic nerve supply.

The latent period between injection of endotoxin and demonstrable sensitization of pulmonary vasoconstrictor mechanisms to injected AC suggested either a slow penetration of endotoxin to the receptor site of AC or the slow formation of metabolites that produced the sensitization to AC.

Doses of endotoxin corresponding to one LD_5 were sufficient to sensitize the pulmonary vasoconstrictor mechanisms to AC (Table I). However, injection of AC in doses as small as 0.1 μg/kg produced a marked rise in RVP after administration of endotoxin. The concentration of AC in the blood after injection of such small doses approached that of AC normally present in the blood and serum of man and rabbits(9,10). Considering the low concentration of AC and the low doses of endotoxin required to produce the observed phenomenon, it is conceivable that endotoxin, or metabolites resulting from injection of endotoxin, sensitize the cholinergic mechanisms to AC that is normally present in the blood or tissues. This may play a role in the pathogenic mechanism of endotoxin shock. Such a hypothesis finds support in the observation that a spontaneous sustained rise in RVP can occur after administration of endotoxin alone(1), with a latent period comparable in duration to that observed before sensitization of pulmonary vasoconstrictor mechanisms to AC.

Rats and guinea pigs, which are more resistant to the lethal action of endotoxin than rabbits(11,12,13), did not respond with increased RVP to injected AC after administration of endotoxin, although the doses of AC were 5 to 10 times and the doses of endotoxin 1000 to 2000 times larger than those injected into rabbits. Experiments are in progress to analyze the mechanism of the observed phenomenon and to establish its significance in the pathogenesis of endotoxin shock in rabbits.

Summary. *Pseudomonas pseudomallei* endotoxin induced in rabbits, after a latent period, an increased response of pulmonary cholinergic vasoconstrictor mechanisms to intravenous injection of acetylcholine. This effect was not found either in rats or guinea pigs. An increased response of cholinergic vasodilator mechanisms in the major circulation to AC was not clearly demonstrated. Cardiac rate and rhythm, central venous pressure, portal pressure and resistance in the respiratory passages were not significantly affected by injection of AC either before or after administration of endotoxin. Endotoxin did not alter the response to stimulation of parasympathetic nerves in any of the cholinergic structures investigated. Only low doses of endotoxin and AC were required to produce the observed phenomenon. The possible role of endotoxin in sensitizing cholinergic mechanisms to AC in the pathogenesis of endotoxin shock in rabbits is discussed.

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