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Blood Pressure Changes in Mice after Lethal Staphylococcal Infection and Endotoxin Challenge[1] (38865)

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Mice injected intraperitoneally with 10^9 *Staphylococcus aureus* (Smith strain) die in 270 ± 20 min after a generalized convulsion (1). Previous studies have shown a rise in blood pCO_2, a fall in blood pH, and a marked congestion in the visceral organs. These changes suggest the presence of cardiovascular shock (2). To clarify these results, blood pressure changes in mice were studied during lethal staphylococcal infections. For comparison, the blood pressure changes after lethal endotoxin challenge in mice were also determined.

Hicks, Giltinan, and Fye (3) recently described a radioisotope tail-cuff technique for the determination of systolic blood pressure in mice. Modifying this technique, we measured the blood pressure changes occurring in mice during the course of staphylococcal infection and after lethal endotoxin challenge. Blood pressures were also measured directly and by the isotope technique in rats to verify the validity of the isotope technique.

Methods. White male mice (Sutter Farms, Springfield, MO) 4–6 wk old and weighing 25–35 g were used. For comparison between strains, male Swiss Albino mice (closed colony, Radiation Research Laboratory, University of Iowa) of the same weight and age were employed. Systolic blood pressure measurements from normal mice were interspaced between all treatment determinations to insure proper experimental control.

Staphylococcal infection was obtained by inoculating 10^9 *Staphylococcus aureus* (Smith strain) after which the mice died in 270 ± 20 min (1). Mice injected with 1.5 mg of endotoxin (Difco Laboratories: Lipo-polysaccharide B, *E. coli*: 055:B5) had an 80% mortality within 30 hr. Sedation was accomplished by injecting 0.05 mg trifluoperazine (Stelazine; Smith, Kline, and French) 30 min prior to blood pressure measurement. All injections and inoculations were made via the peritoneum.

The technique of systolic blood pressure measurement consisted of the detection of radioactivity in the tail while deflating a small latex rubber cuff encircling the base of the tail. The inflatable cuff was attached to a clinical mercury (Hg) sphygmomanometer and a radionuclide ($NaH_2{}^{32}PO_4$) was injected intraperitoneally. The tail was immediately excluded from the circulation by inflation of the cuff causing occlusion of the caudal artery. The cuff was slowly deflated after allowing time for systemic equilibration of the radioisotope. When radioactivity appeared in the tail, the reading was recorded as the systolic blood pressure.

Twenty microcuries of $^{32}P(NAH_2{}^{32}PO_4$; Abbott Labs) in 0.1 ml was injected intraperitoneally via a 27-gauge hypodermic needle. Immediately after the injection, the mouse was placed on one side of a ⅛-in. lead shield; and the tail was threaded through the cuff mounted in the shield. From the other side, the tail was positioned in a Lucite mount and was held gently between the finger tips while the cuff was quickly inflated to 160 mm Hg pressure. The mice, under a small wooden housing, remained quiescent without sedation.

Positioned over the tail, a Geiger-Mueller (G-M) tube served as radiation detector and was connected to a Nuclear Chicago model 1620A count rate meter. A 0.5-sec time con-

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TABLE I. SYSTOLIC BLOOD PRESSURE (±1 SEM) IN MM HG OF NORMAL MICE COMPARING STRAIN AND SEDATION. DETERMINATIONS WERE PERFORMED BOTH DURING THE SUMMER AND WINTER MONTHS (SEE TEXT FOR DISCUSSION).

Strain	Treatment	Summer Room temp.	Winter	
			Room temp.	Warmed
Sutter Farms				
	Control	109 ± 1 (87)[a]	80 ± 4 (20)	101 ± 1 (94)
	Sedated[b]	119 ± 7 (6)		94 ± 2 (24)
Inbred Swiss Albino				
	Control		102 ± 5 (6)	108 ± 1 (16)

[a] Number of animals in parentheses.
[b] Trifluoperazine, 0.05 mg.

stant with a full-scale deflection of 1000 cpm was used.

Our technique varied from that of others (3) in that we used ^{32}P (a beta ray emitter) rather than ^{125}I (a gamma ray emitter) and G-M tube rather than a scintillation crystal for detection.

The manometer pressures and count rates were recorded on a rectilinear strip-chart recorder (Texas Instruments, Inc.) run at a speed of 3 in. per minute. The earliest significant rise in radioactivity was taken as the indication of systolic blood pressure.

Systolic blood pressure was measured by the indirect tail technique in rats simultaneously with direct arterial pressure measurements in a catheterized femoral artery. The direct measurements were recorded through a pressure transducer (Stantham, P23A) fed into a polygraph (Gibson Medical Electronics, model M5P). The pressure transducer connected to the femoral catheter was calibrated against the mercury manometer before each pressure determination.

Measurements were taken on a "summer group" of mice during June through mid-September and on a "Winter group" during November and December. During the summer, the temperature of the room where the experiments were conducted ranged from 25° to 35° while the room temperature during the winter was nearer to 21°.

In the winter experiments, the mice were maintained in a 35° to 37° for 10 min prior to blood pressure measurement maintained with a 250-W Westinghouse "Heat Ray" infrared lamp positioned 50 cm above an open cage.

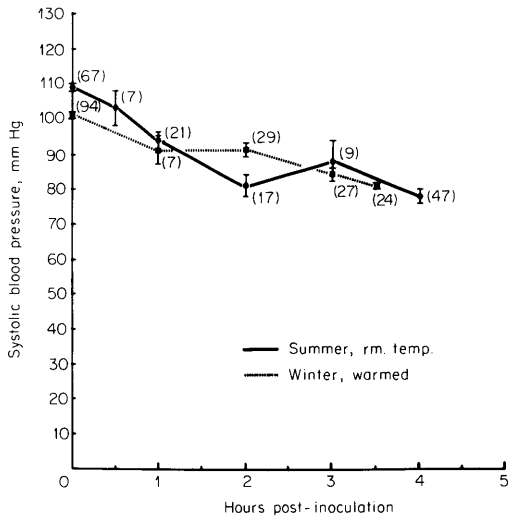

FIG. 1. Mouse systolic blood pressure after staphylococcal infection. Values are means ± 1 SEM and enclosed in parentheses are the number of animals upon which the mean is based.

Blood for all determinations was obtained from torso blood flow immediately after decapitation.

Results. The mean systolic blood pressure value in 87 normal Sutter Farms' mice was 109 ± 1 mm Hg (± SEM) with a range of 79–140 in the summer group and in 91 normal animals in the winter warmed group was 101 ± 1 mm Hg with a range of 71–123. The unwarmed winter group is also shown (Table I).

One hour after *S. aureus* injection a significant fall in blood pressure progresses to a mean of 78 ± 2 mm Hg with a range of

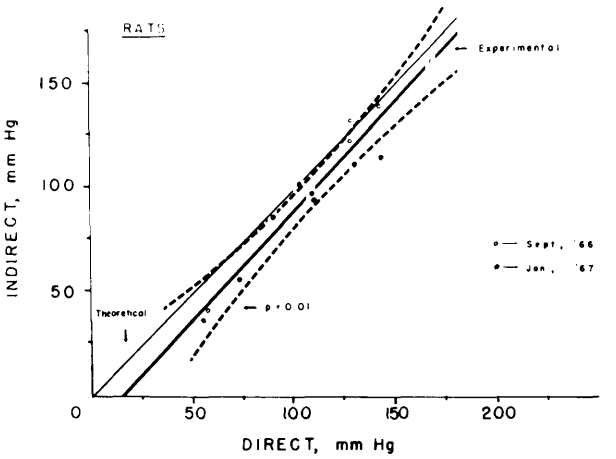

FIG. 2. A comparison of direct systolic blood pressure measurement to that determined by the radioisotope tail-cuff technique. The experiments were performed on rats.

TABLE II. SYSTOLIC BLOOD PRESSURE IN MICE AFTER INTRAPERITONEAL INTRODUCTION OF ENDOTOXIN. VALUES REPRESENT MEANS ±1 SEM AND ARE IN MM HG.

Time Postinoculation of endotoxin	Summer (room temp.)	Winter (warmed)
Control	109 ± 1 (87)[a]	101 ± 1 (94)
4–7 hr	82 ± 5 (15)	83 ± 2 (21)
15 hr	88 ± 4 (25)	78 ± 2 (23)

[a] Number of animals in parentheses.

43–114 at 4 hr postinoculation, i.e., just prior to death (Fig. 1).

The systolic blood pressure dropped within 5 hr after endotoxin challenge (Table II) with no further change in blood pressure evident at 15 hr, just prior to death. In rats the indirect pressure determinations are plotted as a function of the direct measurements (Fig. 2). The 1% confidence interval of the experimental regression line fails to overlap the ideal one-to-one correspondence line only in a narrow region around the mean of the 13 experimental points. The mean difference between the two sets of values was 11 ± 9 (ISD) mm Hg with the indirect pressure measurements giving higher readings. The slope of the experimental regression line is 1.06 ± 0.16, not significantly different from that of the ideal one-to-one correspondence line.

Discussion. The 25-mm Hg lowering of systolic pressure in mice after *S. aureus* injection supports previous findings suggesting stagnation of blood flow (2). As expected, endotoxin challenge also leads to a fall in blood pressure in mice.

In their study of lethal staphylococcal infection in rabbits, Robson and Cluff (4) found that the infection lasted for approximately 24 hr. In comparison to our 4½-hr infection in mice, their rabbit findings can be equated at 4, 12, and 16 hr with our mice findings at 1, 2, and 3 hr. Using this system, their levels run 5–10 mm Hg lower than ours in a parallel manner. Their control readings, however, were 18 mm Hg lower than the levels found in mice. The two studies qualitatively support each other.

The direct measurement of blood pressure in rats with simultaneous measurement by the radioisotope technique confirms the validity of the latter procedure.

In conclusion, we feel that the radioisotope tail-cuff technique is quite reliable and uncomplicated. Of the methods presently available it provides the simplest means of obtaining systolic blood pressure in mice without introducing artifacts. If hypothermia exists, however, warming is necessary. By this method, a 25-mm Hg decrease in systolic blood pressure has been detected in mice after intraperitoneal staphylococcal infection and endotoxin challenge. This may contribute to the pathophysiology of both processes.

Summary. A radioisotope technique has

been used to determine blood pressure changes in mice after lethal staphylococcal infection and after lethal endotoxin challenge. The method was verified by making simultaneous direct measurements in rats. Mice in both groups became hypotensive to a similar level (a fall 20–30 mm Hg). This tail-cuff technique is simple and reliable but is dependent upon normal tail blood flow. Spurious low pressure readings are obtained in hypothermic or chilled mice because the tail is a major thermoregulator organ. These difficulties can be overcome by warming chilled or hypothermic mice.

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