# Direct Blood Pressure Measurements in Rats during Abrupt Exposure to, and Removal from, Cold Air (43686)

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*Abstract.* **When male rats, each bearing an indwelling femoral arterial cannula, were exposed abruptly to cold (5°C), systolic, diastolic, and mean blood pressures increased within 1 hr to levels significantly above those measured prior to exposure to cold. Blood pressures reached maximal levels within 2 hr of exposure to cold and remained elevated during the next seven days. Heart rates, measured at the same time, responded similarly. When cannulated rats that had been exposed to cold for four weeks were removed abruptly to 26°C, systolic, diastolic, and mean blood pressures decreased nearly linearly for 5 hr. By two days after removal from cold, blood pressures stabilized at levels that were still elevated above those observed prior to exposure to cold. In contrast, heart rate returned to precold exposure level by the 13th day after removal from cold. The mechanisms responsible for the elevation of blood pressure during abrupt exposure of rats to cold and the decrease in blood pressure after removal from cold remain to be elucidated.** [P.S.E.B.M. 1994, Vol 205]

It is now well established that chronic exposure of rats to mild cold (5°C) induces hypertension, including elevation of systolic, diastolic, and mean blood pressures and cardiac hypertrophy (1–5). When assessed by indirect measurement of systolic blood pressure from the tail, the first significant elevation of blood pressure occurs within two to three weeks of exposure to cold (1–5). This technique, however, is less sensitive in detecting an elevation of blood pressure than measurement of blood pressure directly (1). We reported previously an elevation in mean blood pressure of 20 mm Hg after 1 hr of exposure to cold (1). Wasserstrum and Herd (6), using adult squirrel monkeys, reported that mean blood pressure increased an average of 24 mm Hg after 1 hr of exposure to cold (10°C). Other investigators have also reported an increase in the blood pressure of humans (7, 8) and lambs (9, 10) during acute exposure to cold, although Popovic *et al.* (11) reported no change in the rat. Since long-term exposure to cold is now well known to elevate blood pressure in the rat, it was deemed worthwhile to assess the immediate and short-term effect of exposure to cold on blood pressure and heart rate measured directly. When these measurements were completed, direct blood pressures and heart rates were measured daily for seven days in the same animals while they remained in the cold. In a second group of rats kept in the cold for four weeks, blood pressures were measured continuously upon removal of the rats from cold and at intervals for 23 days thereafter. The results of these measurements are described below.

## Materials and Methods

Two separate experiments were carried out. Each used male rats of the Harlan Sprague-Dawley strain (Harlan Industries, Indianapolis, IN) weighing initially 200–250 g. The rats were housed individually in temperature-controlled rooms maintained at 26 ± 2°C during the control period and at 5 ± 2°C during the experimental period. All rats received Purina Laboratory Chow (#5001, Ralston Purina Co., St. Louis, MO) to

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eat and tap water to drink *ad libitum*. The rooms were illuminated from 7:00 AM to 7:00 PM daily.

To carry out this study, all rats had their left femoral artery cannulated while anesthetized with sodium pentobarbital (40 mg/kg, ip). The cannula was made of polyethylene (PE50) and contained heparinized saline (10 IU/ml). After cannulation of the artery, the cannula was tunneled beneath the skin to exit at the nape of the neck. It was sealed with a stainless steel stylette. Each cannula was flushed daily with heparinized saline. Systolic, diastolic, and mean blood pressures, as well as heart rate, were measured by means of a pressure transducer (Statham P23dB) and recorded on a Grass polygraph. During all measurements, the rats were unanesthetized.

**Experiment 1. Changes in Systolic, Diastolic, and Mean Blood Pressures during Acute and Chronic Exposure to Cold.** The femoral artery in each of six rats was cannulated as described above. The rats were allowed to recover for three days. On the fourth day, beginning at 9:00 AM, a 0.5-hr equilibration period began. At 9:30 AM blood pressures and heart rates were measured directly at 15-min intervals for 0.5 hr prior to exposure to cold. Blood pressures and heart rates were then measured at 15-min intervals for the first 3 hr of exposure to cold. Thereafter, blood pressures and heart rates were measured daily for seven days excepting only Days 4 and 5 which fell on a weekend.

**Experiment 2. Time-Course of Change in Systolic, Diastolic, and Mean Blood Pressures upon Removal from Cold.** Six naive rats were used. During a 2-week control period, systolic blood pressures were measured indirectly from the tail once weekly using the method described by Fregly (12) and a NarcoBio Instruments Co. polygraph. At the end of this control period, the rats were exposed to cold (5°C) where they remained for 10 weeks. Systolic blood pressure was measured weekly to assure that blood pressures had become elevated during exposure to cold. By the end of the 10th week in the cold, the average systolic blood pressure was 155 ± 5 mm Hg. At this time, the rats were removed from cold and the left femoral artery cannulated as described above. All rats were allowed two days to recover and were then returned to cold. One week later, after establishing baseline data for systolic, diastolic and mean blood pressures, the rats were removed from cold to 26°C beginning at 10:00 AM. Systolic, diastolic, and mean blood pressures were measured directly thereafter at 1-h intervals for 3 hr.

Twenty-four hours after removal from cold, blood pressures and heart rates were measured in all rats. They were again measured on Days 2, 13, and 23 following removal from cold.

Statistical analysis of the data in both Experiments 1 and 2 was made by means of a one-way ANOVA for comparison of precold with cold (Experiment 1) and cold with postcold (Experiment 2) measurements. The pooled variance from the analysis of variance was used to assess the significance of the difference between measurements. Significance was set at the 95% confidence limit.

## Results

**Experiment 1.** When the rats were exposed abruptly to cold air, systolic, diastolic, and mean blood pressures increased rapidly within the first 45 min, and continued to increase at a slower rate for the remainder of the study (Fig. 1). The first significant ($P < 0.05$) rise in systolic, mean, and diastolic blood pressures compared with precold exposure pressures occurred within 30 min of exposure to cold. Differences from precold exposure blood pressures remained significant ($P < 0.01$) thereafter for the duration of the experiment. By 45 min, systolic, mean, and diastolic blood pressures reached levels of 150, 122, and 111 mm Hg, respectively, and remained at these levels for the duration of the 3-hr study (Fig. 1) and for the next 21 hr; that is, Day 1 of exposure to cold (Fig. 2A). During subsequent days in cold air, systolic, mean, and diastolic pressures gradually increased above those observed after one day of exposure to cold to reach levels of 163, 133, and 122 mm Hg, respectively, by seven days of exposure to cold (Fig. 2A). Heart rate showed a similar response but reached a maximum of approximately 530 bpm by the second day of exposure to cold. This level was maintained reasonably constant throughout the remainder of the 7-day exposure to cold (Fig. 2B).

**Experiment 2.** When rats that had been exposed to cold for 10 weeks were abruptly removed from cold, systolic, mean, and diastolic blood pressures de-

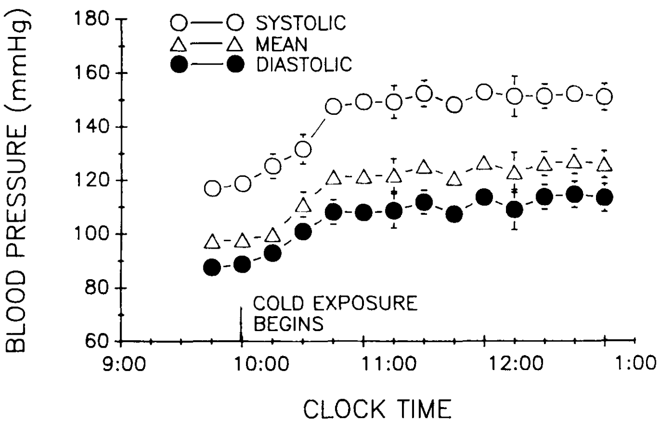


**Figure 1.** Systolic, diastolic, and mean blood pressures of six unanesthetized rats during exposure to cold air (5°C). The rats were placed in the cold at 10:00 AM. Means ± SE are shown. The symbols identifying the three blood pressures are shown in the figure. When SE is not shown, it falls within the symbol.

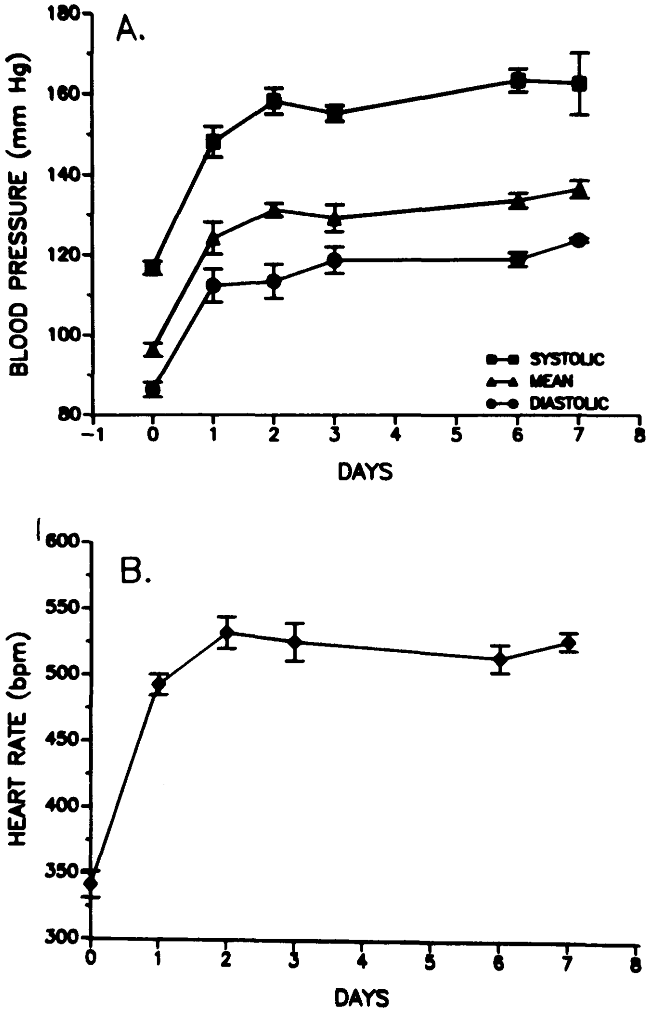


**Figure 2.** (A) Systolic, diastolic, and mean blood pressures of six unanesthetized rats measured at intervals during a 7-day exposure to cold (5°C) are shown. The rats are the same as those in Figure 1. (B) Mean heart rate (±SE) is shown. The symbols identifying the three blood pressures are given in panel A.

creased nearly linearly for 5 hr (Fig. 3). The first significant ($P < 0.05$) decrease in systolic, mean, and diastolic pressures, compared with blood pressures measured prior to removal, occurred within 2 hr after removal from cold. By the first day after removal from cold, blood pressures had decreased significantly ($P < 0.01$) and became less variable (Fig. 4A). Blood pressures tended to be lower through the 13th day after removal from cold, but were increased slightly on the 23rd day. Although blood pressures decreased after removal from cold, they did not reach the precold exposure levels observed in the rats of Experiment 1 (see Fig. 1), even at 23 days after removal from cold (Fig. 4A). Heart rate declined within the first day of removal from cold and continued to decline sharply on the second day (Fig. 4B). A gradual decrease continued until the 13th day, after which heart rate remained approximately constant and similar to the precold exposure heart rate observed in Experiment 1 (see Fig. 1).

## Discussion

The results of the first study reveal the greater sensitivity of direct, compared with indirect, blood pressure measurements in determining the time required for an elevation of blood pressure in rats exposed abruptly to cold. Indirect measurement of systolic blood pressure in earlier experiments suggested that the first significant elevation occurs within two to three weeks of exposure to cold (1–5). Direct measurement of blood pressure in the first study indicated that mean blood pressure became elevated significantly within the first hour of exposure to cold. Blood pressure, as well as heart rate, increased abruptly during the first week of exposure to cold and was nearly maximal by the third week (Fig. 2A).

The mechanism responsible for the elevation of blood pressure on exposure to cold is not elucidated by this study. The increase in heart rate induced by abrupt exposure to cold suggests that an increased secretion of catecholamines, especially norepinephrine, has occurred. Indeed, exposure to cold for one day increases significantly both plasma concentration and urinary output of norepinephrine in cold-treated rats (4, 13). It may be speculated that an increased secretion of norepinephrine contributes by triggering an increased secretion of renin, and subsequently angiotensin 2. The latter may be responsible for the elevation of blood pressure, either alone or in combination with norepinephrine (14). Attempts have recently been made to assess pharmacologically the validity of this speculation and have revealed that agents that block the renin-angiotensin-aldosterone system at various levels can either prevent or attenuate the development of cold-induced hypertension (15–17).

The results of the second study show that blood pressure does fall immediately after removal from cold (Fig. 3). On the first day after removal from cold, blood pressure appears to have fallen to its lowest level. It later rebounds to slightly higher levels where stabilization occurs. Even after 23 days at 26°C, systolic, mean, and diastolic blood pressures were still elevated above those observed in warm-acclimated rats (Fig. 1). An earlier study in which rats were exposed to cold for five weeks and systolic blood pressure was measured indirectly from the tail also showed a decrease in blood pressure after removal from cold (2). After four weeks in air at 26°C in the present study, systolic blood pressure of cold-treated rats also failed to reach the level observed prior to exposure to cold. This suggests that a 10-week exposure to cold (5°C), as used in the present study, induces an elevation of blood pressure either by a mechanism(s) that does not easily reverse upon removal from cold or by physiological and morphological changes (*e.g.*, hypertrophy of the walls of resistance vessels) that are not

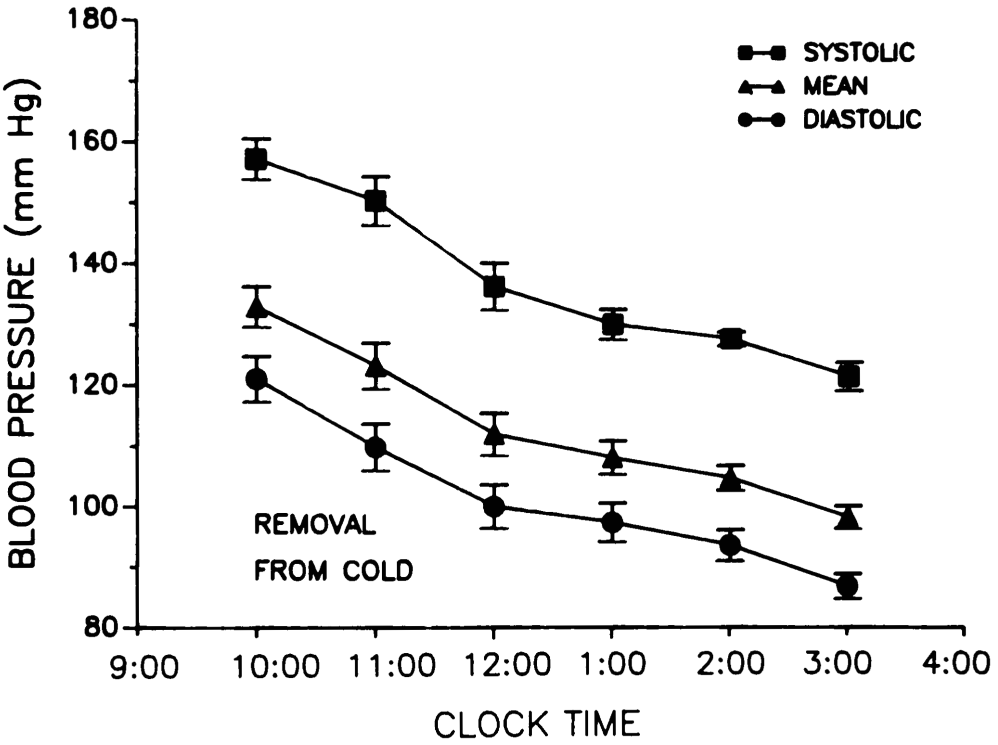


**Figure 3.** Systolic, diastolic, and mean blood pressures of six unanesthetized rats following removal from cold (5°C) to 26°C. Blood pressures were measured hourly beginning at 10:00 AM. Means ± SE are shown. The symbols identifying the three blood pressures are shown in the figure.

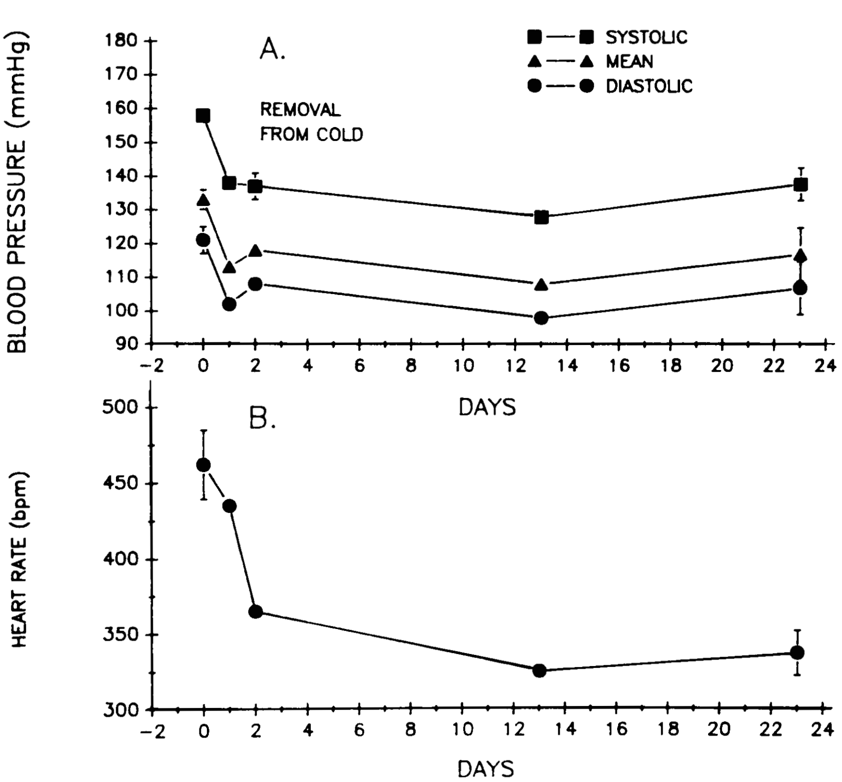


**Figure 4.** (A) Systolic, diastolic, and mean blood pressures of six unanesthetized rats measured at intervals after removal from cold (5°C) to 26°C. The rats are the same as those shown in Figure 3. (B) Heart rate of the same rats is shown. Means ± SE are shown. When SE is not shown, it falls within the symbol. The symbols identifying the three blood pressures are shown in the figure.

readily reversible upon removal from cold. Whether a longer period of exposure to cold would result in a permanent, irreversible elevation of blood pressure upon removal from cold remains to be determined.

Little is known of the physiological mechanisms that are responsible for the reduction in blood pressure after removal from cold. We showed earlier that urinary output, and presumably plasma concentration, of norepinephrine returned to control level within one day after removal from cold. The elevated heart rate accompanying exposure to cold was reduced significantly by the second day after removal from cold. Heart rate returned to the level of rats not previously exposed to cold by the thirteenth day after removal from cold (Fig. 4B). Hence, while the reduced rate of secretion of norepinephrine correlates directly with heart rate of rats removed from cold, it seems unlikely that it maintains the elevation of blood pressure after removal from cold. Other factors, such as an elevation in the rate of secretion of the renin-angiotensin system, a failure to produce adequate amounts of endothelium-derived relaxing factor (*i.e.*, nitric oxide), and others, may play a role. Additional studies will need to be carried out to assess these possibilities.

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