

Effect of Environmental Hypothermia on Vitelline Artery Blood Pressure and Vascular Resistance in the Stage 18, 21, and 24 Chick Embryo

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ABSTRACT. We studied the effect of environmental hypothermia on arterial blood pressure, dorsal aortic blood flow, and vascular resistance in stage 18, 21, and 24 chick embryos. The arterial pressure was measured with a servo-null micropressure system. Mean dorsal aortic blood flow was calculated from pulsed-Doppler measurement of mean dorsal aortic blood velocity and dorsal aortic diameter. Vascular resistance was calculated by dividing mean vitelline arterial blood pressure by dorsal aortic blood flow. Sequential data were obtained at temperatures of 34.7, 31.1, and 34.1° C. At stage 21, the vitelline arterial blood pressure decreased from 0.82 ± 0.03 ($\bar{x} \pm \text{SEM}$) to 0.72 ± 0.03 mm Hg on cooling and increased from 0.66 ± 0.05 to 0.87 ± 0.06 mm Hg on rewarming ($p < 0.05$). At stage 21, mean dorsal aortic blood flow decreased from 0.49 ± 0.02 to 0.33 ± 0.02 mm³/s with cooling and increased from 0.34 ± 0.02 to 0.47 ± 0.02 mm³/s with rewarming. The vascular resistance in stage 21 embryos increased after cooling from 1.68 ± 0.19 to 2.23 ± 0.39 mm Hg/mm³/s ($\bar{x} \pm 95\%$ confidence interval). The changes were similar in stage 18 and 24 embryos. We conclude that the reduction of vitelline artery blood pressure resulted from a decrease in cardiac output. In addition, we noted that the vitelline arterial vascular bed can constrict in response to hypothermia prior to autonomic innervation. These changes in hemodynamics may be a teratogenic mechanism for hypothermia-induced cardiac defects in the chick embryo. (*Pediatr Res* 19:651-654, 1985)

that blood pressure and cardiac output decreased and vascular resistance increased with hypothermia in all stages.

METHODS

Fertile white Leghorn chicken eggs were incubated blunt end up in a forced draft constant humidity incubator to Hamburger and Hamilton (3) stages 18 (approximately 3 days), 21 (3½ days), and 24 (4 days). We opened the shell and removed the outer and inner shell membranes to expose the embryo. We inserted a 5- μ m diameter-tipped drawn glass micropipette electrode in a first-order vitelline artery for measuring pressure with a servo-null micropressure system (model 900, WP Instruments). We previously showed this technique is linear over a range of 0-30 mm Hg ($y = 0.995x - 0.23$, $r^2 = 0.99$, $\text{SEE} = 0.11$ mm Hg) (4). Environmental temperature was monitored with a 1-mm thermistor probe placed adjacent to the embryo. Since it was not technically possible to measure embryo body temperature directly, we assumed that environmental temperature accurately reflected embryo core temperature.

Mean dorsal aortic blood flow, an index of cardiac output, was determined by measuring mean dorsal aortic blood velocity with a 20 MHz pulsed-Doppler velocity meter. The 1-mm piezoelectric crystal was positioned over the dorsal aorta at a 45° angle. We previously showed that this technique is linear over a range of 0-16 mm/s ($y = 0.85x + 1.15$, $r^2 = 0.99$, $\text{SEE} = 0.49$ mm/s) (4). Dorsal aortic diameter was measured with a filar micrometer eyepiece calibrated against a 10- μ m scribed glass standard. Mean dorsal aortic blood flow was calculated as the product of mean blood velocity and aortic area. Vascular resistance was calculated by dividing mean vitelline artery blood pressure by dorsal aortic blood flow.

After baseline recording at a temperature of 34.7° C, the egg was exposed to cool air for approximately 5 min until the environmental temperature reached 31.1° C. We then recorded blood pressure and heart rate. The egg was then rewarmed by warm air for approximately 5 min to a temperature of 34.1° C.

The experimental recordings were divided into two parts. Arterial pressure was recorded in a group of embryos during cooling and a separate group of embryos during rewarming. This was necessary to avoid alteration of the baseline when hypertonic electrolyte solution diffused within the micropipette shifting the resistance of the system.

Since duplicate individual measurements were not performed on the same embryo, mean and 95% confidence limits for vascular resistance were determined by ratio analysis of the mean and SD of pressure and flow at each stage (5). Blood pressure, mean dorsal aortic blood flow, and heart rate data are presented

Low environmental temperature is one of the hazards to the chick embryo during incubation. In experimental studies, a decrease in incubation temperature causes embryo death or malformations of various organs including the heart (1). Hypothermia also results in hemodynamic changes including a decrease in heart rate and dorsal aortic blood flow in the embryo (2). There are, however, no integrated studies on the effects of hypothermia on blood pressure and vascular resistance.

We studied the effect of hypothermia on vitelline artery blood pressure, dorsal aortic blood flow, and vascular resistance in the stage 18, 21, and 24 chick embryos, points in development prior to the functioning of the autonomic nervous system. We found

Received May 2, 1984; accepted February 20, 1985.

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Supported by NIH Grant HL-14388. E.B.C. is the recipient of NIH Research Career Development Award HD-00376.

as mean \pm SEM. We used a paired *t*-test to analyze these results. Probabilities of less than 5% ($p < 0.05$) are presented as statistically significant.

RESULTS

Mean vitelline artery blood pressure decreased significantly on cooling and returned to baseline measurements with rewarming at each stage (Fig. 1; Table 1). Mean dorsal aortic blood flow also decreased significantly with cooling and returned to baseline with rewarming (Fig. 2). Heart rate decreased on cooling but did not completely return to baseline on rewarming (Fig. 3).

Vascular resistance increased significantly with cooling at each stage studied (Fig. 4). With rewarming, the vascular resistance in stage 21 and 24 embryos showed a decreasing trend but increased slightly in stage 18 embryos.

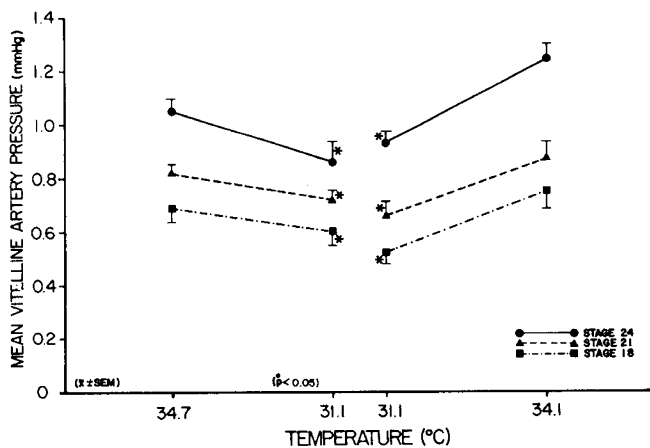


Fig. 1. Mean vitelline artery pressure versus environmental temperature for experimental embryo at stages 18, 21, and 24. Separate groups of embryos were studied for the response to cooling and rewarming (see text). Asterisks indicate statistically differences ($p < 0.05$) from initial temperature.

DISCUSSION

We are studying the functional characteristics of the chick embryo cardiovascular system. We previously described the changes in cardiac output, heart rate, and vascular resistance that occur as the embryo develops (4). As a natural extension of these studies, we sought to define the embryos response to environmental temperature changes.

Since the chick embryo is poikilothermic, it is important to determine the cardiovascular response to a decrease in environmental temperature, a naturally occurring risk during incubation. Previous investigators have shown that low environmental temperature decreases heart rate in the chick embryo (6) as well as in cultured heart cells (7). We reported that the decrease in embryo heart rate results in a decrease in dorsal aortic blood flow, an index of cardiac output. However, stroke volume remains unchanged (2). We speculated that this response is a protective mechanism to compensate for the effect of temperature fluctuation during incubation.

In order to have a comprehensive picture of the embryos response to hypothermia, it is important to know what changes occur in arterial pressure and resistance. Girard (8) showed that

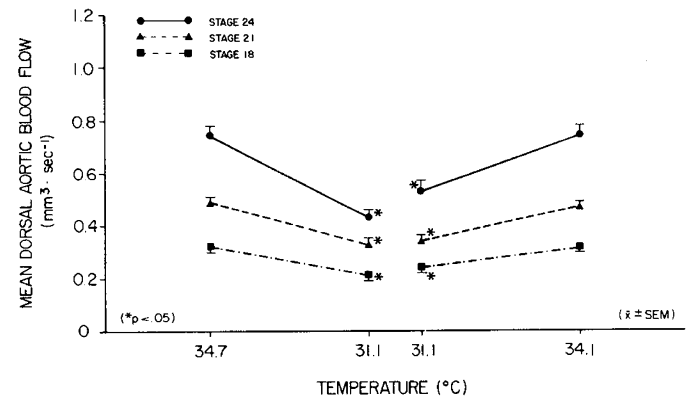


Fig. 2. Mean dorsal aortic blood flow versus environmental temperature for experimental embryos at stages 18, 21, and 24.

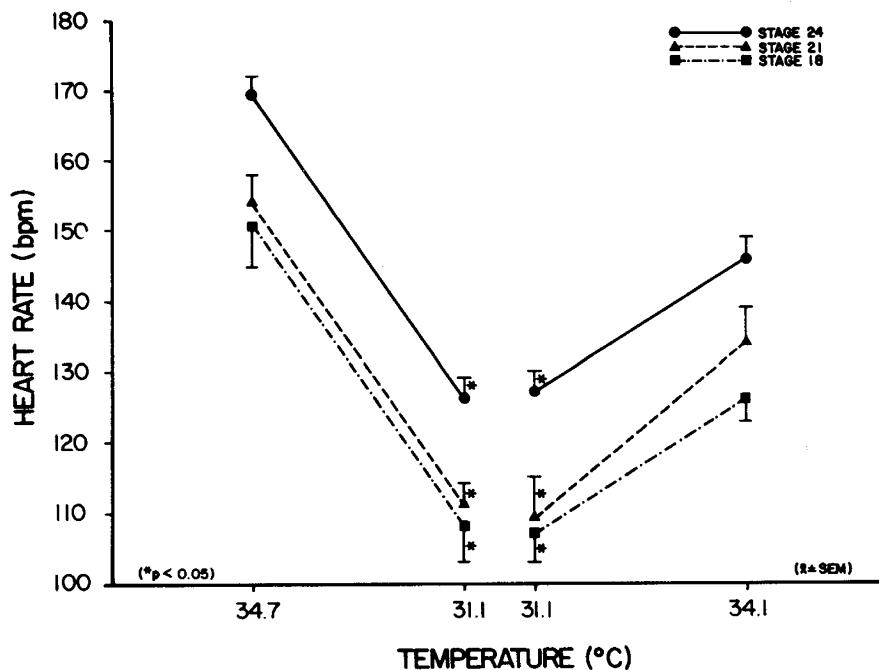


Fig. 3. The change in heart rate observed among stage 18, 21, and 24 experimental embryos following cooling from 34.7 to 31.1° C and after rewarming from 31.1 to 34.1° C.

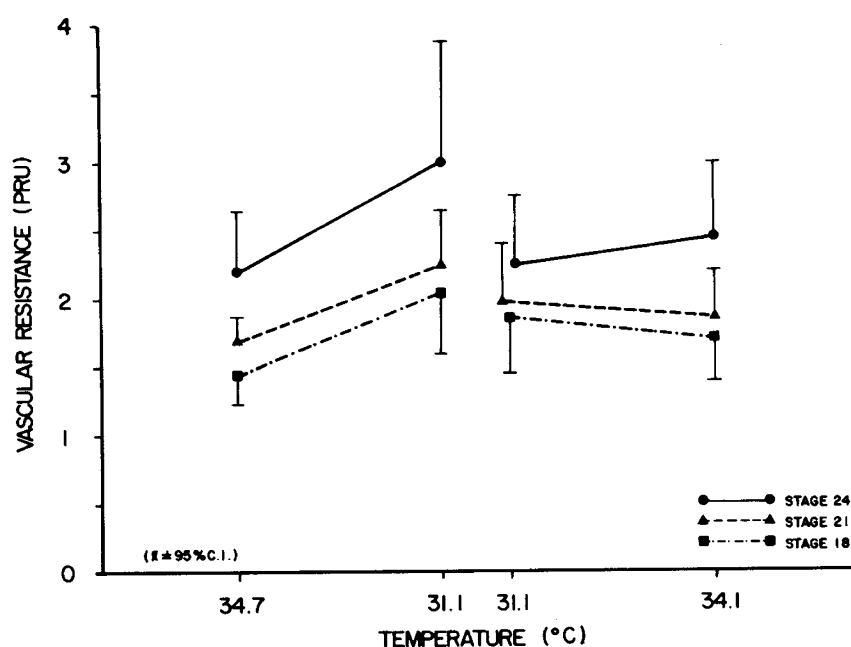


Fig. 4. Mean vascular resistance (\pm 95% confidence interval) versus environmental temperature for experimental embryos at stages 18, 21, and 24.

Table 1. Heart rate, mean vitelline artery pressure, mean dorsal aortic blood flow, and vascular resistance by stage at each of the environmental temperatures (mean \pm SEM)

	Stage	34.7° C	31.1° C	31.1° C	34.1° C
Heart rate (bpm)	18	151 \pm 6	108 \pm 5	107 \pm 4	126 \pm 3
	21	154 \pm 4	111 \pm 3	109 \pm 6	134 \pm 5
	24	169 \pm 3	126 \pm 3	127 \pm 3	146 \pm 3
Mean vitelline artery pressure (mm Hg)	18	0.69 \pm 0.05	0.60 \pm 0.05	0.52 \pm 0.04	0.75 \pm 0.06
	21	0.82 \pm 0.03	0.72 \pm 0.03	0.66 \pm 0.05	0.87 \pm 0.06
	24	1.05 \pm 0.04	0.86 \pm 0.07	0.93 \pm 0.04	1.24 \pm 0.06
Mean dorsal aortic blood flow (mm ³ /s)	18	0.32 \pm 0.02	0.21 \pm 0.02	0.24 \pm 0.02	0.31 \pm 0.01
	21	0.49 \pm 0.02	0.33 \pm 0.02	0.34 \pm 0.02	0.47 \pm 0.02
	24	0.74 \pm 0.04	0.43 \pm 0.03	0.53 \pm 0.04	0.74 \pm 0.04
Vascular resistance (mm Hg/mm ³ /s) (\bar{x} \pm 95% confidence interval)	18	2.18 \pm 0.43	3.00 \pm 0.86	2.22 \pm 0.53	2.44 \pm 0.53
	21	1.68 \pm 0.19	2.23 \pm 0.39	1.98 \pm 0.42	1.86 \pm 0.32
	24	1.44 \pm 0.23	2.04 \pm 0.45	1.81 \pm 0.37	1.71 \pm 0.30

arterial blood pressure decreased in both the 3- and 7-day chick embryo in response to hypothermia. Warbanow (9) demonstrated a similar response in embryos at 16 days of incubation. However, in neither of these studies was cardiac output measured and consequently vascular bed resistance could not be determined.

In the current study, we measured the effect of environmental cooling on arterial blood pressure, cardiac output index, and calculated vascular resistance. We confirmed that arterial blood pressure in stage 18, 21, and 24 chick embryos decreased in response to environmental hypothermia. Our calculations indicate that the chick embryo compensates for a decrease in cardiac output by increasing vascular resistance at stages 18, 21, and 24. The subsequent return of vascular resistance toward baseline following rewarming suggests that this response is reversible and likely a mechanism important in cardiovascular regulation of the primitive circulation.

Maintenance of blood pressure would preserve perfusion of primitive embryonic blood vessels that supply the rapidly developing organs. The persistence or disappearance of vascular channels such as the aortic arch is dependent on blood flow and pressure which maintain vessel patency (10).

We expect that the resistance is modulated in the extraembryonic vascular bed. The vascular plexus of the embryonic circulation is very primitive and devoid of smooth muscles at these stages while the extraembryonic vascular bed is more histologically complex.

In mature animals, a change in peripheral resistance is a protective mechanism. In mammals with a functional autonomic nervous system, hypothermia causes peripheral vasoconstriction through both direct action on the resistance vessels and an increase in sympathetic tone. The response to a cool environment compensates for a decrease in cardiac output (11). Our study in the chick embryo, suggests that an increase in vascular resistance is a very primitive response that compensates for environmental hypothermia prior to autonomic innervation.

At stages 18 to 24, the autonomic nervous system is probably not functional. Functional parasympathetic and sympathetic innervation of the heart occurs at stages 38 and 41, respectively (12, 13). The time of functional innervation of the extraembryonic vascular bed has not yet been defined. However, anatomic intervention of extraembryonic vasculature is derived from the prevertebral plexus which forms during the 5th and 6th days

of incubation and postganglionic nerves are found in the peritoneal cavity by 10 days (14). Therefore, it is unlikely that the extraembryonic vascular is innervated during the period we studied.

The chick embryo's vascular bed is dynamic. Although the adrenergic nervous system is not functional, adrenergic receptors are present in the heart and vascular system. This is not surprising since adrenergic-like receptors are phylogenetically primitive and are present in single cell organisms presumably as a mechanism for intracellular communication (15). In a chick embryo, β receptors have been identified in the myocardium at 2.5 days of incubation and are functional at 5 days of incubation (16). We found that isoproterenol caused a paradoxical increase in vascular resistance mediated through a β receptor (17, 18). In addition to receptor-mediated response, we found that caffeine, a phosphodiesterase inhibitor, decreased vascular resistance in chick embryos at stage 24 (19).

The increase in vascular resistance in response to hypothermia may be mediated by circulating neurohumoral agents. Wallace (20) found that the embryo produces serotonin as early as stage 10. In addition, egg yolk contains epinephrine and norepinephrine (21). Although there is no adrenal gland at stages 18 to 24, a change in environmental temperature may alter the vascular sensitivity to neurohumoral agents that are already present. In the mature duck, resistance vessel's sensitivity to norepinephrine increases 2-fold as the temperature is reduced from 35 to 20° C (22). Thus, hypothermia may increase resistance vessel response to a steady state circulating level of a vasoactive drug.

The change in vascular resistance may also be related to the critical closing pressure of the vascular bed. As mean arterial pressure decreases below the critical closing pressure, resistance vessels collapse. This phenomena may explain the resistance change at these stages. Resistance vessel diameter may increase with development: thus, the critical closing pressure will be highest in stage 18 embryos, accounting for the observed changes on rewarming.

These observations may also be important to the understanding of the teratogenic effects of hypothermia. In the chick embryo, a transient reduction in incubation temperature increases the mortality rate and the incidence of cardiac defects in the surviving embryos (1). Although temperature may affect the rate of cell development and cell death, our study defined a profound change in cardiovascular physiology. Numerous indirect investigations have suggested that alteration of cardiac rheology by mechanical interference or drugs leads to a production of cardiac anomalies (23). We speculate that an alteration in blood flow patterns and blood vessel wall stress is an important factor in the pathogenesis of hypothermic-induced cardiac defects.

Acknowledgments. Thanks are due to Dr. Atsuyoshi Takao for encouraging this collaborative project, Sue Kucera for preparation of the manuscript, and Horst Jordan for the graphics.

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