# Thermoregulatory and Cardiac Responses of Infant Spontaneously Hypertensive and Wistar-Kyoto Rats to Cold Exposure

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*Abstract*—Cardiovascular function during cold exposure is dependent on effective thermoregulation. This dependence is particularly apparent in infants. For example, we have previously demonstrated that in infant rats during cold exposure, cardiac rate is directly related to their ability to produce heat endogenously. The primary source of endogenous heat production for infant rats is brown adipose tissue (BAT). Because of the dependence of cardiac rate on effective thermoregulation in the cold and because hypertension in spontaneously hypertensive rats (SHR) is influenced by the preweanling environment, in this study we examined the thermoregulatory and cardiac rate responses of infant SHR and Wistar-Kyoto rats (WKY) to varying levels of cold exposure. In experiment 1, 7- to 8-day-old SHR and WKY were acclimated at a thermoneutral air temperature (35°C) and then exposed to successive decreases in ambient temperature (30.5°C, 26.5°C, 23°C, and 17°C) while thermal and metabolic measures were recorded. Although both strains increased BAT thermogenesis and oxygen consumption in response to cold exposure, SHR cooled more than WKY and exhibited lower levels of oxygen consumption at the lowest air temperatures. Experiment 2 was identical to experiment 1 except that cardiac rate was also measured. Again, SHR exhibited substantial thermoregulatory deficits compared with WKY; in addition, they were less able than WKY to maintain cardiac rate at the 2 lowest air temperatures tested. Finally, in experiment 3, infant SHR exhibited diminished BAT thermogenesis in response to a range of doses of a selective $\beta_3$-adrenoceptor agonist. We hypothesize that long-term thermoregulatory deficits during the early postnatal period influence cardiovascular function and contribute to the development of hypertension in SHR. (***Hypertension*. 1999;33:1465-1469.**)

**Key Words:** body temperature regulation ■ brown fat ■ nonshivering thermogenesis ■ heart rate ■ rats, inbred strains ■ hypertension, experimental

The degree of high blood pressure expressed in adult spontaneously hypertensive rats (SHR) is influenced by the neonatal environment.[1–3] Thus, manipulations limited to the preweanling period, such as cross-fostering,[2] pharmacological interventions,[1,4] and handling,[5,6] result in the lowering of blood pressure in adult SHR. Such findings highlight the importance of epigenetic processes in the development of established hypertension in SHR.

We have recently demonstrated that thermoregulatory mechanisms play an important role in the protection of cardiovascular function during cold exposure in infant rats.[7–9] Specifically, heat produced by brown adipose tissue (BAT) during moderate cold exposure helps to warm cardiac muscle and by doing so contributes to the maintenance of cardiac rate. In contrast, pronounced bradycardia is produced by either extreme air temperatures that overwhelm the ability of BAT to deliver warm blood to cardiac muscle or ganglionic blockade that prevents the activation of BAT thermogenesis in response to the cold.

The ability of BAT thermogenesis to protect cardiac rate during moderate cooling and the loss of this function in response to further decreases in ambient temperature have important implications for cardiovascular control in the infant rat. Because cardiac rate serves as the primary mechanism for the control of cardiac output in infant mammals,[10] bradycardia in response to cooling would be associated with either a drop in arterial pressure or an increase in vascular resistance to maintain pressure. Recordings of arterial pressure from infant rats during cold exposure suggest that the latter possibility is more likely, because blood pressure is maintained at basal levels during both moderate and extreme ambient temperatures.[8] Thus, it seems that nonshivering thermogenesis buffers against bradycardia during moderate cooling, but as cardiac rate decreases during extreme cooling, vascular resistance must be increased to maintain blood pressure.

Despite the accepted influence of the preweanling environment on the development of hypertension in SHR, the

contribution of thermal factors has not been examined. Therefore, the present study was performed to determine whether thermoregulatory and cardiac responses of infant SHR to cold exposure differ from those of their normotensive control, the Wistar-Kyoto rat (WKY). We found that SHR are more sensitive than WKY to cold challenge, exhibiting deficiencies in their ability to maintain cardiac rate as air temperature decreases. This deficiency of SHR may be the result of a number of factors, including reduced body size and, as shown here, diminished BAT thermogenesis. These results suggest that infant SHR are more susceptible to episodes of acute hypothermia and bradycardia that may, as a result, contribute to their development of hypertension.

## Methods

### Experimental Subjects

Infant male WKY and SHR, born to breeding stock received from Taconic Farms (Germantown, NY) and maintained in our laboratory, were used for the present studies. On the day after birth (day 1), litters were culled to 8 pups and litters with fewer than 6 pups were excluded from the study. All pups were 7 to 8 days old on the day of testing. For experiment 1, 8 WKY pups from 8 litters and 8 SHR pups from 8 litters were used. For experiment 2, 5 WKY pups from 5 litters and 5 SHR pups from 5 litters were used. For experiment 3, 40 WKY pups from 12 litters and 40 SHR pups from 13 litters were used, with littermates always assigned to different experimental groups. All animals were maintained in polypropylene cages (48×20×26 cm) with wood shavings as bedding material. Food and water were available ad libitum and a 12:12-hour light-dark cycle was maintained with lights on at 6 AM.

### Procedures

The procedures used in the present study have been fully described elsewhere[7] and are only briefly presented here. For experiment 1, an individual pup was removed from a litter on the day of testing, weighed, and placed in an incubator maintained at 35°C to 36°C. Two chromel-constantan thermocouples (Omega) were then secured to the skin surface with collodion as an adhesive. One thermocouple was placed above the BAT in the interscapular region ($T_{is}$), and the second was placed in the lumbar region ($T_{back}$) distant from the BAT. For experiment 2, pups were lightly anesthetized with ether ($\approx$1-minute exposure), and then 3 ECG leads were implanted transcutaneously and secured with collodion. Thermocouples were then placed on the pups as in Experiment 1.

For testing, an individual pup was placed inside a double-walled glass metabolic chamber (height=17.5 cm; ID=12.5 cm) maintained at 35°C, a thermoneutral air temperature for 1-week-old rats.[11] Air temperature in the chamber ($T_a$) was controlled by circulation of temperature-controlled water from a water bath through the walls of the chamber. Animals were allowed a 45-minute adaptation period before baseline measurements were acquired for 10 minutes. $T_a$ was then successively decreased to 30°C, 27°C, 23°C, and 17°C, with 45 minutes between each decrease.

Data were acquired with a customized data acquisition system. Thermocouple signals underwent cold-junction compensation before acquisition by the computer. Oxygen consumption ($\dot{V}_{O_2}$) was determined with a 2-channel electrochemical oxygen analyzer that compared the oxygen concentration of the airstream passing to the chamber with the exhaust airstream passing from the chamber. The percent difference in oxygen concentration between these 2 airstreams was computed to 0.001% and entered in the data acquisition system which computed oxygen consumption in mL $O_2 \cdot$ kg body wt$^{-1} \cdot$ min$^{-1}$. For both experiments, thermal and oxygen consumption measurements were recorded twice per minute during the test session.

For experiment 2, the ECG signal was amplified and filtered before it was digitized by a second data acquisition system. The ECG

analog signal was digitized at a rate of 1000 samples per second, and interbeat intervals (IBI) were determined continuously online with a customized software program with a peak threshold detector (this method provides results identical to direct measurement of intervals between successive R waves).[9] IBI data were acquired by the computer at a rate of 30 samples per minute.

For experiment 3, thermocouples were attached in the interscapular and lumbar regions, and the pup was placed in a temperature-controlled chamber to acclimate for 45 minutes at 35°C. After this acclimation period, data collection began for a baseline period of 5 minutes, after which the pup was injected with 0, 0.1, 1, 10, or 100 mg/kg of the selective $\beta_3$-adrenoceptor agonist (CL-316243–2; donated by Wyeth-Ayerst, St Davids, Pa) dissolved in isotonic saline. Pups were injected subcutaneously with a volume of 1 $\mu$L/g body wt. Data collection continued for 60 minutes with $T_a$ maintained at 35°C.

### Data Analysis

Thermal, metabolic, and cardiac data were imported into StatView 4.5 for the Macintosh. For each pup in experiments 1 and 2, a single score for each variable at each air temperature was determined from measurements taken during the final 2 minutes at each temperature. For experiment 2, cardiac rate values in bpm were determined from the interbeat interval for each pup during this same time period. Repeated-measures ANOVA was used to test for differences in the variables between strains and across time. Post hoc *t* tests were used for follow-up comparisons; paired *t* tests were used to compare successive air temperatures within a strain and unpaired *t* tests to compare the strains at each air temperature.

For experiment 3, the maximal change from baseline in $T_{is}$ and $T_{is} - T_{back}$ was determined for each pup during the 60-minute test. A 2-factor ANOVA was used to test the main effects of strain and dose on these variables, and post hoc *t* tests were used to compare the 2 strains at each dose.
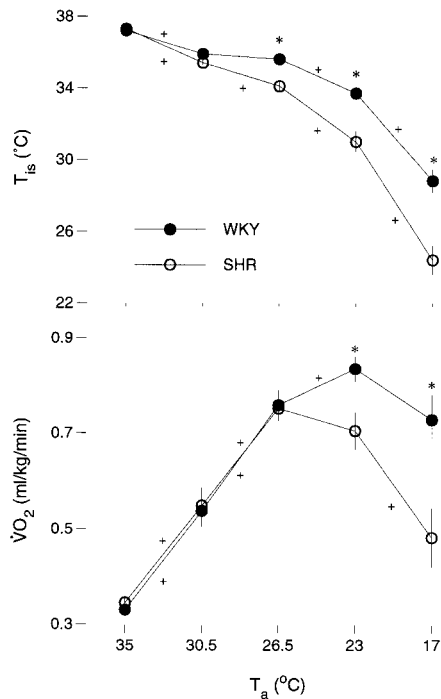
For all tests, the $\alpha$ level was set at 0.05, and the Bonferroni adjustment was used to correct for multiple post hoc comparisons. All data are presented as mean±SEM.

## Results

Body weights were significantly different between strains in experiment 1 (WKY: 17.8±1.2 g; SHR: 12.4±0.9 g; $t_{14}$=3.5, $P<0.005$), experiment 2 (WKY: 19.8±0.4 g; SHR: 12.1±0.3 g; $t_8$=15.3, $P<0.0001$), and experiment 3 (WKY: 22.3±0.4 g; SHR: 13.6±0.5 g; $t_{78}$=14.0, $P<0.0001$).

Figure 1 presents $T_{is}$ and $\dot{V}_{O_2}$ for the 1-week-old WKY and SHR in experiment 1. The $T_{is}$ responses to cold exposure were significantly different between infant WKY and SHR ($F_{1,13}$=30.7, $P<0.0001$). At the thermoneutral $T_a$ of 35°C, $T_{is}$ did not differ between the strains, but as $T_a$ decreased, $T_{is}$ decreased further in SHR than in WKY ($F_{4,52}$=10.2, $P<0.0001$). Similarly, the $\dot{V}_{O_2}$ responses to cold exposure differed between the 2 strains ($F_{1,14}$=5.7, $P<0.05$). Although both strains showed progressive increases in $\dot{V}_{O_2}$ as $T_a$ decreased, the WKY pups showed a continued increase in oxygen consumption as $T_a$ decreased to 23°C and maintained oxygen consumption at the $T_a$ of 17°C ($F_{4,56}$=6.4, $P<0.0005$). These differences in $\dot{V}_{O_2}$ reflect differences in heat production between the 2 strains and are consistent with the greater ability of WKY to maintain $T_{is}$ during cold exposure.

Figure 2 presents $T_{is}$ and $\dot{V}_{O_2}$ for the infant WKY and SHR in experiment 2. Overall, the thermal and metabolic responses of pups in experiment 2 mirrored those found for pups in experiment 1. Specifically, $T_{is}$ differed significantly between the strains ($F_{1,8}$=28.1, $P<0.001$), with greater decreases in SHR at the lower $T_a$s ($F_{4,32}$=47.6, $P<0.0001$). For $\dot{V}_{O_2}$,
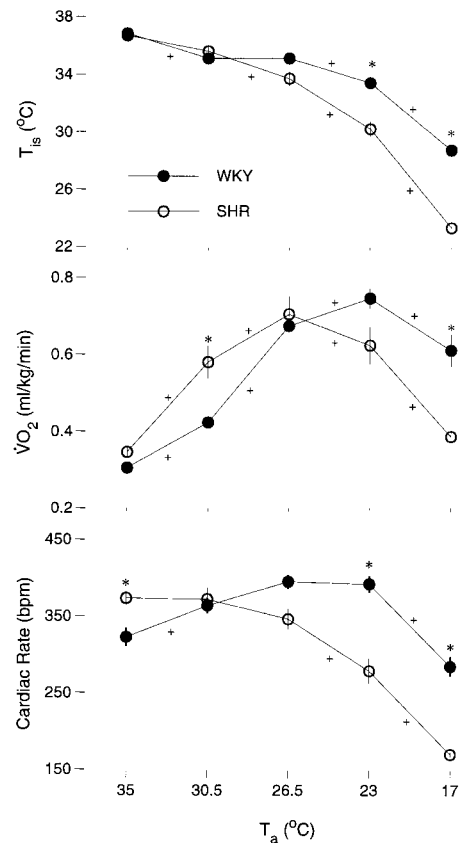
**Figure 1.** $T_{is}$ and $\dot{V}_{O_2}$ at a thermoneutral $T_a$ and during successive decreases in $T_a$ for the 1-week-old WKY and SHR in experiment 1. Infant WKY were better able to increase and maintain thermogenesis during cold exposure than were infant SHR. n=7 to 8 per group. *$P<0.01$ by unpaired $t$ test. +$P<0.0125$, paired $t$ test.



**Figure 2.** $T_{is}$, $\dot{V}_{O_2}$, and cardiac rate (bpm) at a thermoneutral $T_a$ and during successive decreases in $T_a$ for the 1-week-old WKY and SHR in experiment 2. As in experiment 1, infant WKY were better able to increase and maintain thermogenesis during cold exposure than were infant SHR and, in addition, they were better able to maintain cardiac rate at the 2 coldest $T_a$s. n=5 per group. *$P<0.01$ by unpaired $t$ test. +$P<0.0125$, paired $t$ test.

although there was not a significant main effect of strain ($F_{1,8}=0.5$), there was a significant strain×$T_a$ interaction ($F_{4,32}=25.1$, $P<0.0001$). Although both strains showed significant increases in $\dot{V}_{O_2}$ in response to the initial decrease in $T_a$, there was a more pronounced decrease in $\dot{V}_{O_2}$ for SHR at the lowest $T_a$.

Cardiac rate for the infant WKY and SHR in Experiment 2 are also presented in Figure 2. There was an overall difference in cardiac rate between WKY and SHR ($F_{1,8}=15.6$, $P<0.005$). Cardiac rate of SHR was elevated at the thermoneutral $T_a$ compared with WKY, consistent with previous findings.[12] The 2 strains, however, showed markedly different cardiac rate responses to cooling ($F_{4,32}=36.3$, $P<0.0001$): whereas WKY increased or maintained cardiac rate down to a $T_a$ of 23°C, the cardiac rate of SHR declined steadily, such that at the $T_a$ of 23°C, cardiac rate had fallen significantly and was lower than that of WKY.

Because infant rats cannot shiver,[13] increases in $\dot{V}_{O_2}$ during cold exposure reflect heat production by BAT. In addition to $\dot{V}_{O_2}$, increases in the differential between $T_{is}$ (measured adjacent to the interscapular BAT) and $T_{back}$ (measured at a site distant from the interscapular BAT) reflect selective heat production by interscapular BAT as well as heat retention within the thoracic cavity. Figure 3 presents this differential, $T_{is}-T_{back}$, plotted against $T_a$, for the infant WKY and SHR in experiments 1 and 2. These plots indicate an increase in values of $T_{is}-T_{back}$ with a decrease in $T_a$, suggestive of an increase in BAT thermogenesis. In addition, the best-fit polynomial regressions, consistent with the data in Figures 1 and 2, indicate higher values of $T_{is}-T_{back}$ for WKY than SHR
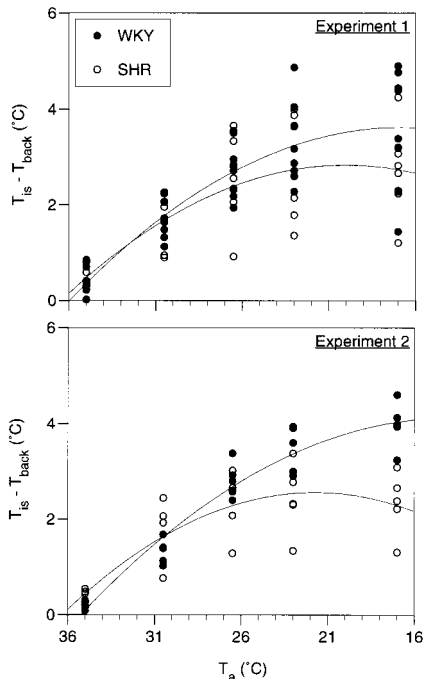
in both experiments. These strain differences should be interpreted with caution, because the larger body size of the WKY may have influenced its magnitude. Regardless, the patterns of the regression lines are generally consistent with the conclusion that, although both WKY and SHR increased BAT thermogenesis in the cold, WKY were better able to maintain this heat production at the lowest $T_a$s tested here.

The dose-related thermogenic responses of infant SHR and WKY to the selective $\beta_3$ adrenoceptor agonist in experiment 3 are presented in Figure 4. The left panel depicts the maximum changes in $T_{is}$ to the drug during each 60-minute test. A 2-factor ANOVA indicated significant main effects of dose ($F_{4,70}=85.8$, $P<0.0001$) and strain ($F_{1,70}=44.8$, $P<0.0001$), as well as a significant interaction ($F_{4,70}=7.6$, $P<0.0001$). The right panel depicts the maximum changes in $T_{is}-T_{back}$ during each 45-minute test. Again, a 2-factor ANOVA indicated significant main effects of dose ($F_{4,70}=48.8$, $P<0.0001$) and strain ($F_{1,70}=19.8$, $P<0.0001$), as well as a significant interaction ($F_{4,70}=4.8$, $P<0.005$).

## Discussion

At a thermoneutral air temperature, cardiac rate was elevated in 1-week-old SHR compared with 1-week-old WKY, al-

**Figure 3.** The differential between interscapular and back temperatures ($T_{is}-T_{back}$) vs $T_a$ for infant WKY and SHR in experiments 1 and 2. Increases in $T_{is}-T_{back}$ reflect the compartmentalization of heat within the thoracic cavity, in part due to the production of heat by the interscapular BAT. Polynomial regression lines through the data indicate that as $T_a$ decreased, $T_{is}-T_{back}$ increased for all pups in both experiments and, in addition, these increases were smaller for the infant SHR. These results are consistent with the thermal and metabolic data presented in Figures 1 and 2 and suggest a decreased capacity for BAT thermogenesis in infant SHR.
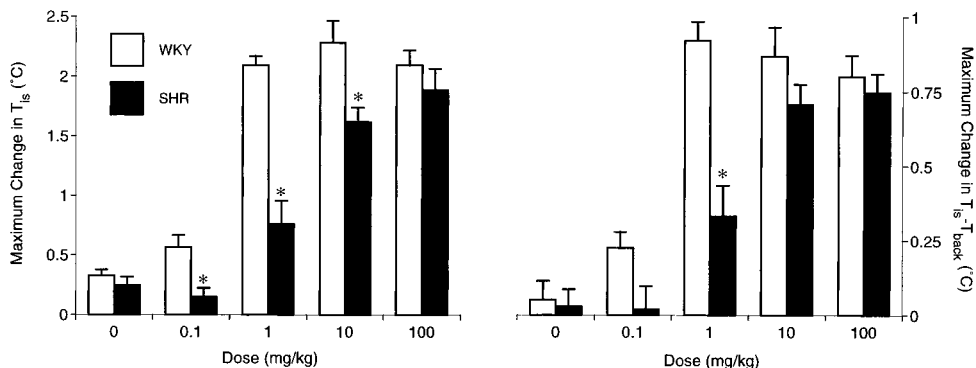
though thermal and metabolic measures were equivalent between the strains. In contrast, during cold challenge, SHR were deficient in their thermoregulatory capabilities, with the strain differences becoming more pronounced at the lowest air temperatures tested. Thus, SHR exhibited lower values of $T_{is}$ and oxygen consumption at air temperatures of $\leq 23°C$. These decreased values of $T_{is}$ suggest a diminished capability of SHR to deliver warmed blood to cardiac muscle,[7,9] thus leading to the pronounced bradycardia seen in experiment 2.

The diminished thermoregulatory capabilities of infant SHR during cold exposure may be due to exaggerated heat

loss and/or insufficient heat production. Because of their smaller size, SHR have larger surface-to-volume ratios versus age-matched WKY, which results in increased heat loss.[14] Although the smaller body size of SHR places them at a thermoregulatory disadvantage versus WKY, it may also be true that SHR are deficient in their ability to use BAT to produce heat, as has been shown in adult rats after central injections of prostaglandin $E_2$.[15] Indeed, the results of experiment 3 provide direct support for this possibility in that selective activation of BAT with a $\beta_3$-adrenoceptor agonist produced diminished thermogenesis in infant SHR versus age-matched WKY. This diminished thermogenesis was evident at moderate doses of the $\beta_3$-agonist. In contrast, equivalent thermogenesis between the 2 strains was found at the highest dose administered. In other words, the dose response curve of the infant SHR was shifted to the right. These results are consistent with a decrease in sensitivity of the $\beta_3$ adrenoceptor population in BAT of infant SHR, although other factors acting between receptor activation and thermogenesis may be involved. Therefore, these results suggest that both the ability to produce and the ability to retain heat in the cold make the isolated infant SHR more susceptible to hypothermia-induced bradycardia than the isolated WKY.

When the dam is out of the nest, infant rats huddle with littermates, a behavior that results in increased heat retention and metabolic savings in the cold.[16] Although the benefits of huddling to each individual pup are considerable, huddling does not replace the need for endogenous heat production at typical colony room air temperatures (G.S., M.S.B., M.M. Adams, written observations, 1999). This raises the question of whether the thermoregulatory deficiencies of individual SHR translate into thermoregulatory deficiencies of huddling SHR. Again, research in our laboratory suggests that this is possible: 1-week-old golden hamsters lack the ability to produce heat endogenously and gain little thermoregulatory advantage from huddling (G.S. and M.S.B., unpublished data, 1998). Thus, even huddling SHR are probably experiencing more frequent and more severe thermal challenges than huddling WKY.

The present results do not support an increased level of sympathetic drive that controls BAT thermogenesis during either basal conditions (ie, thermoneutral) or in response to cold challenge. Similarly, Smith[17] found no evidence of



**Figure 4.** Maximal change in $T_{is}$ and maximal change in the differential between interscapular and back temperatures ($T_{is}-T_{back}$) after injection with various doses of the $\beta_3$ adrenoceptor agonist, CL-316243–2, for the WKY (open bars) and SHR (solid bars) pups in experiment 3. n=8 per group. *Significantly different from WKY, $P<0.01$, unpaired $t$ test.

increased sympathetic tone of the levator palpatris muscle in developing SHR versus WKY. These results stand in marked contrast to those that concern sympathetic control of cardiovascular function in preweanling SHR. For example, sympathetic drive of the vasculature is elevated in anesthetized infant SHR, and pressor responses to the sympathetic agonist, methoxamine, are exaggerated.[18] Increased sympathetically mediated pressor responses have also been demonstrated in unanesthetized preweanling SHR.[19] The pressor response of SHR pups to suckling is double that of WKY, and the response can be eliminated by ganglionic blockade. In addition, greater sympathetic influences on cardiac regulation are found in infant SHR.[12,20,21] Together, these results suggest that there is not a generalized increase in sympathetic drive of peripheral tissues in SHR during the preweanling period but that the level of sympathetic tone is tissue-specific, with a selective increase in the drive of tissues associated with the cardiovascular system.

Long-term exposure of adult rats to an air temperature of 5°C leads to the development of hypertension within 1 to 2 weeks.[22] Given this finding in adults, the effects of long-term cold exposure and other factors on hypertension would be expected to be much greater in infant rats that are engaged in the process of calibrating various physiological systems.[3] For an infant SHR, bouts of acute cold exposure that produce hypothermia and bradycardia would necessitate increases in peripheral resistance to maintain arterial blood pressure.[8,10] If the hypothermia and bradycardia were experienced repeatedly, then repeated increases in peripheral resistance during early development could reduce vascular compliance in the adult, perhaps contributing to the development of hypertension. Therefore, thermal factors during development, in addition to a myriad of other physiological,[1] maternal,[2] and dietary factors,[23,24] may contribute to the development of hypertension in SHR.

## Acknowledgments

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