Suppression of the Sweat Gland Sensitivity to Acetylcholine Applied Iontophoretically in Tropical Africans Compared to Temperate Japanese

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Abstract: Tropical inhabitants possess the ability of heat–tolerance through permanent residence in the tropics. Previously, we had shown that tropical African and Thai subjects regulate the core temperature with less amount of sweat against heat compared to temperate Japanese subjects and that suppression of sweating in tropical subjects was attributed to suppression in both central and peripheral sudomotor mechanisms. To elucidate the peripheral mechanisms of the suppressed thermal sweating in tropical natives, sweating responses to acetylcholine (ACh), a primary transmitter of the sudomotor innervation, were compared between Japanese (20 healthy males) and Africans (10 healthy males). ACh was iontophoretically administered on the forearm. Directly activated and axon reflex–mediated sweat responses were evaluated by quantitative sudomotor axon reflex test. The sweat onset–time was 0.72 min shorter ($P<0.01$) and the sweat volumes were 72% – 110% higher ($P<0.01$) in the Japanese than the Africans. Iodine–impregnated paper method revealed that sweat gland density was 50.6% higher ($P<0.001$) and sweat gland output per single gland was 20.4% larger ($P<0.001$) in the Japanese compared to the Africans. The Japanese showed the a 0.17℃ higher oral temperature and a 0.30℃ higher forearm skin temperature compared to the Africans ($P<0.05$, respectively) at rest under a thermoneutral condition. ACh iontophoresis did not produce any influences on oral temperature, but increased the local skin temperature in both the Japanese and the Africans. These results indicate that suppressed thermal sweating in Africans is, at least in part, attributed to the suppressed glandular sensitivity to ACh through both recruitment of sweat glands and sweat output per each gland.

Key words: Long–term heat acclimatization, Tropical natives, Sweat gland density, Sweat gland output, Quantitative sudomotor axon reflex test.

INTRODUCTION

Physiological acclimatization to ambient heat provides man with an effective thermoregulatory capacity which can prove life saving in extremely hot conditions. Evidence suggests that when unacclimated humans are exposed to temperatures above 30℃, the physiological capacity of thermoregulation may be compromised (Wilkerson et al, 1986). Heat acclimation increases thermal resistance by causing adaptive changes in thermoregulatory mechanisms. Transitory acclimation to heat by repeated exposures to heat and/or physical training has been intensively investigated by many researchers. And it was well accepted that heat–tolerance was achieved by the lowered threshold for sweating and enhanced sweating in short–term acclimation (Nadel et al, 1974; Ogawa and Sugenoya, 1993). On the contrary, tropical inhabitants showed heat–tolerance with suppressed sweating (Kuno, 1956; Ogawa and Sugenoya, 1993; Matsumoto et al, 1993, 1997). Adaptation to temporary exposure to heat and acclimatization to a tropical climate by permanent residence were distinguishable from each other (Kuno, 1956). The thermotolerance with suppressed sweating and enhanced dry heat loss is predominant than that with enhanced sweating which is seen in short–term heat acclimation from the view points of body fluid maintenance and osmoregulation (Matsumoto et al, 1997). It seems probable that human beings acquire acclimatization by an ability to avoid excessive sweating beyond the limits of rational heat regulation. However, the underlying mechanisms of the suppressed sweating in tropical natives remained to be clarified. Heat acclimation is a continuum of temporally varying process shared by both the central nervous system and the peripheral effectors (Horowitz, 1989). Sweating is a powerful heat loss response in human beings, especially those under a extremely hot conditions, and is centrally regulated by preoptic area and anterior hypothalamus (PO/AH), as well as peripherally by the sympathetic postganglionic innervation, where the primary neurograndular transmitter is acetylcholine (ACh). Upward shift of threshold core temperature for sweating and decreased ACh–sensitivity of the sweat glands in Thai subjects (Matsumoto et al, 1997) suggest that sudomotor mechanism is down–regulated both centrally and peripherally in tropical natives.

In order to further elucidate the peripheral mechanism of suppressed sweating in tropical natives, local sweating response as well as sweat gland density activated by ACh applied iontophoretically were compared between permanent residents of tropical Africa and temperate Japan under a thermoneutral condition.

MATERIALS AND METHODS

Subjects

Twenty healthy male Japanese and 10 healthy male Africans volunteered for this study. African subjects (Nigerian, Equatorial Guinea, Tanzanian, Senegalese, Ghanaian, Sudanese, Kenyan and Chadian) were Japan International Cooperation Agency (JICA) trainees and academic researchers who stayed in the Institute of Tropical Medicine, Nagasaki University between October 1997 and January 1998. Japanese subjects were students and staff at

Nagasaki University. Experiments on Africans were conducted within 1–2 weeks of residence in Japan. The physical characteristics of the Japanese were 172.4 ± 4.9 cm in height, 64.90 ± 6.85 kg in weight, 29.9 ± 3.8 years old in age and $1.76 \pm 3.75 m^2$ in body surface area (BSA). Corresponding characteristics for the Africans were 174.0 ± 4.9 cm in height, 72.40 ± 8.55 kg in weight, 31.1 ± 3.7 years old in age and $1.87 \pm 3.75 m^2$ in BSA. There were significant differences in weight ($P < 0.01$) and BSA ($P < 0.05$) when statistically analyzed. Each subject gave his informed consent after being thoroughly acquainted with the purpose and experimental procedures as well as any potential risks. We paid great attention to the subjects in accordance with the Helsinki Declaration of 1975.

Measurements and procedures

All experiments were carried out in a climatic chamber (24 ± 0.5℃, relative humidity $40 \pm 3\%$ and less than 1 m/sec air velocity) at 2–5 p.m. between October 1997 and January 1998 in Nagasaki, Japan. Upon arrival into the climatic chamber, the subject wore light indoor clothing and sat on a chair for 60 min before the experiment.

Quantitative sudomotor axon reflex test, QSART (Low et al, 1983; Kihara et al, 1993; Low, 1997), was performed to quantitatively evaluate glandular ACh–sensitivity. The QSART capsule consists of three concentric compartments. ACh iontophoretically applied stimulates the underlying sweat glands in the outer compartment directly while the glands of the skin in the central compartment of the capsule are activated indirectly via axon reflex. Sweating response was measured from directly activated (DIR) and axon reflex–mediated (AXR) sweat responses resulting from the iontophoresis. Two sets of QSART capsules were attached on the volar aspect of the forearm with rubber bands, one at the mid portion between wrist and elbow joints and another at 10 cm proximal of the former one. The outer compartment of the former capsule was filled with 10 % ACh (Ovisot, Daiichi Pharmaceutical Co., Ltd., Japan) solution. Two mA of direct current was applied for 5 min between an electrode on the ACh cell (anode) and a flexible plate–electrode (HV–BIGPAD, Omron, Kyoto) (cathode) attached on the forearm skin just proximal to the wrist joint. The central compartment of the ACh capsule served as the site for sudomotor axon reflex AXR(1) measurement during the 5 min of iontophoresis. Immediately after the cessation of current loading, sweat capsules were detached, the skin covered with ACh capsule was wiped up and then the two capsules positions were exchanged. This procedure took less than 20 sec. The data was acquired for another 5 min to permit the simultaneous observation of DIR(2) and AXR(2) sweating.

Sweat onset–time, latent period for sweating after current loading, and sweat volume for 5 min, area under the sweating curve, 0–5 min for AXR(1) and 6–11 min for AXR(2) and DIR(2) were used for analysis. Sweat rates were measured by the capacitance hygrometer–ventilated capsule method (Matsumoto et al, 1993). In brief, nitrogen gas flowed into each compartment with a constant flow rate of 0.3 l/min, and the change of the relative humidity of effluent gas was detected by a hygrometer (H211, Technol Seven, Yokohama). Oral (sublingual) and skin temperatures just beside and 10 cm away from the ACh capsule were monitored using thermistors (PXK–67, Technol Seven, Yokohama) connected to data logger

(K–720, Technol Seven, Yokohama). Sweating rates and temperatures were recorded with PC (PC9801, NEC, Japan) every 5 sec.

At the end of QSART recording, the sweat gland density was determined with iodine –impregnated paper method (Sato and Sato, 1983). Iodine–starch paper was placed on the skin which was directly stimulated with ACh for several seconds after wiping up. The shorter duration was selected after preliminary experiments, although Sato and Sato (1983) recommended 15 sec. Blue–black pigment spots in 0.5 cm•0.5 cm areas were counted under a microscope in triplicate, and average sweat gland density (count•cm^{-2}) was calculated. Sweat output per single gland (μg•min^{-1} • gland^{-1}) was finally obtained by division of DIR sweating rate (mg•cm^{-2} • min^{-1}) by the sweat gland density.

Statistical analysis

Values are presented as means ± SD. Statistical significance was assessed by unpaired Student's t–test for comparison between Japanese and Africans, and by one–way ANOVA for repeated measures at the 0.05 level.

<div align="center">RESULTS</div>

Typical recording of a single subject is shown in Fig. 1. When ACh was applied iontophoretically, AXR sweating occurred after a latent period (sweat onset–time) and reached a plateau phase within a few min. After the end of iontophoresis, DIR(2) sweating became sustained, while AXR(2) sweating declined to the baseline during observation. Forearm skin temperature just beside ACh capsule tended to rise following sweat onset. Any changes were not observed in the skin temperatures 10 cm proximal or distal to ACh capsule throughout experiment.

Sweat onset–time was 0.72 min shorter in the Japanese compared to the Africans (Fig. 2). The difference was statistically significant ($P<0.01$).

The AXR(1), AXR(2) and DIR(2) sweat volumes of Japanese were 87%, 110% and 72% greater than the Africans (Fig. 3). The differences were significant, $P<0.01$ for AXR(1) and AXR(2), and $P<0.001$ for DIR(2).

The initial value of oral temperature was 0.17℃ higher in the Japanese compared to the Africans. The differences were statistically significant ($P<0.05$). Oral temperature showed a slight tendency of elevation throughout recording period, however, there was no influence of ACh iontophoresis on oral temperature. Initial value of the forearm skin temperature was also 0.3℃ higher in the Japanese compared to the Africans ($P<0.05$). Skin temperature just beside ACh capsule increased during ACh iontophoresis in both Japanese and Africans (Fig. 4). At the end of iontophoresis, the skin temperature decreased in Africans while it remained high in Japanese.

Sweat gland density (activated sweat glands by ACh iontophoresis) was 50.6% higher in the Japanese compared to the Africans (Fig. 5). The difference was significant ($P<0.001$).

Sweat gland output per single gland was also 20.4% larger in the Japanese compared to the Africans (Fig. 6). The difference was significant ($P<0.001$).
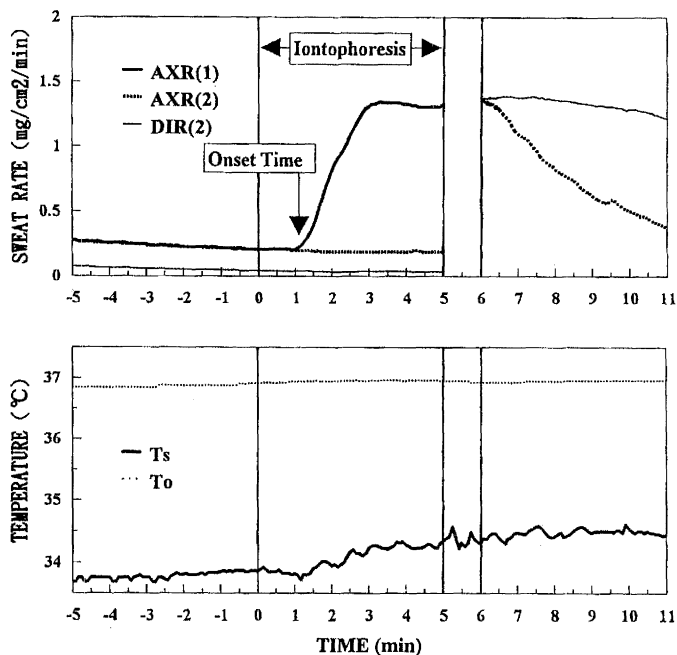
Fig. 1. Typical recording of the AXR(1), AXR(2) and DIR(2) sweating (upper panel) and of oral and skin temperatures (lower panel) in a Japanese subject. Iontophoresis of 10% ACh was performed with 2 mA of direct current for 5 min. Just after the cessation of current loading, the sweat capsules were exchanged each other, then sweating rates recording was continued for another 5 min. Sweat onset–time was 1.08 min. Sweat volume was 3.14 mg•cm^2, 3.15 mg•cm^2 and 5.88 mg•cm^2 on AXR(1), AXR(2) and DIR(2), respectively. Ts: Skin temperature just beside ACh capsule, To: Oral temperature.
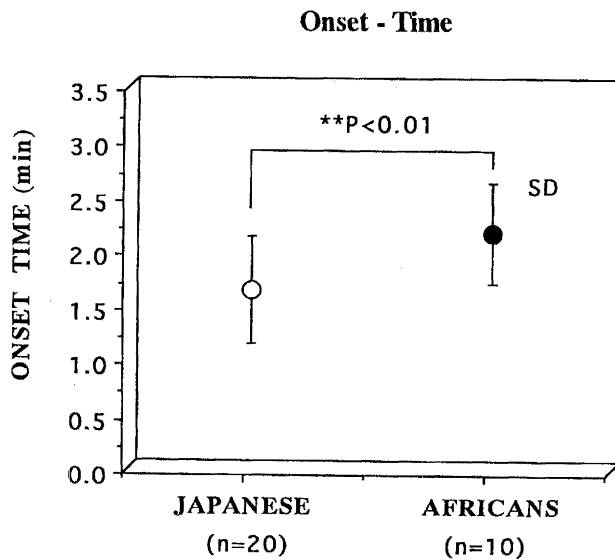
Onset - Time

Fig. 2. Comparison of the sweat onset–time between the Japanese and the Africans.
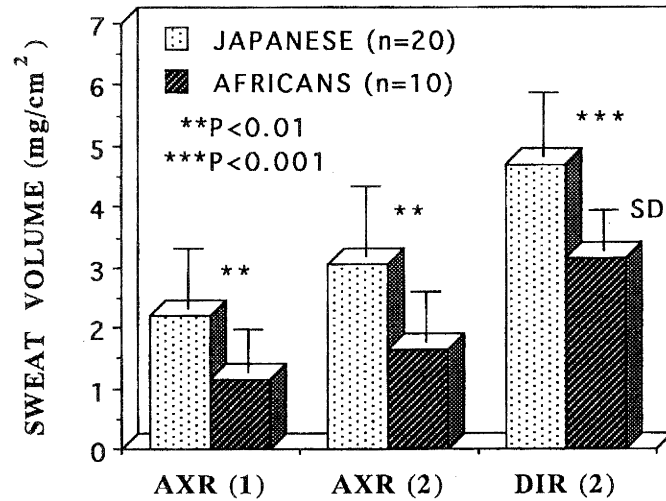
Sweat Volume

Fig. 3. Comparison of the sweat volume induced by ACh applied iontophoretically between the Japanese and the Africans. AXR(1): area under the curve for 0–5 min, AXR(2) and DIR(2): area under the curve for 6–11 min.
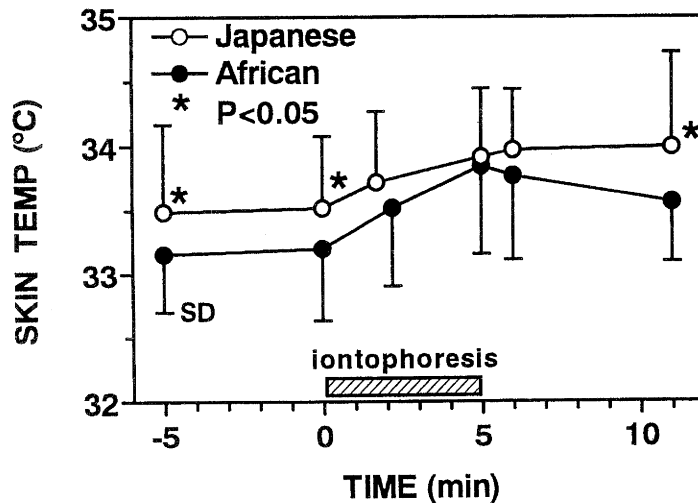

Fig. 4. Changes in skin temperature (mean±SD) just beside the ACh capsule before, during and after iontophoresis and at sweat onset in the Japanese and the Africans. * : P < 0.05, Japanese vs. Africans.
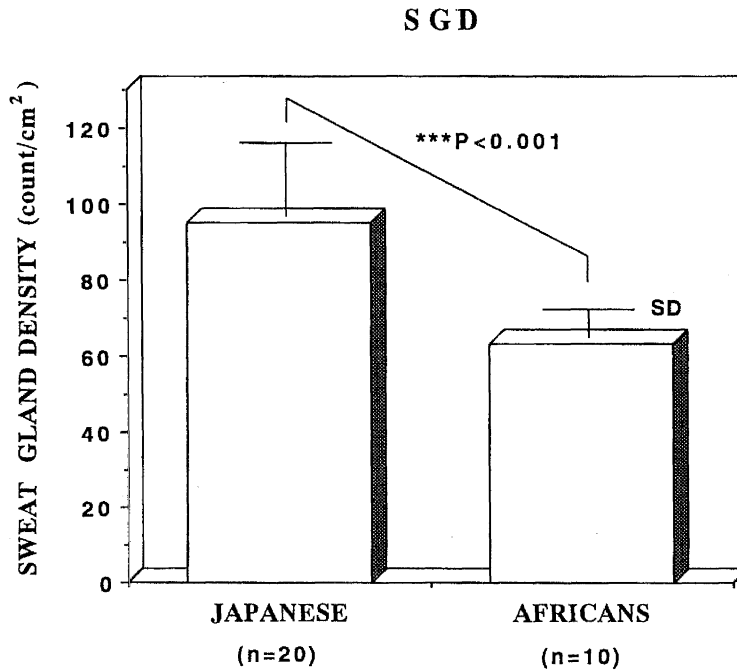
S G D

Fig. 5. Comparison of sweat gland density activated by ACh iontophoresis between the Japanese and the Africans.
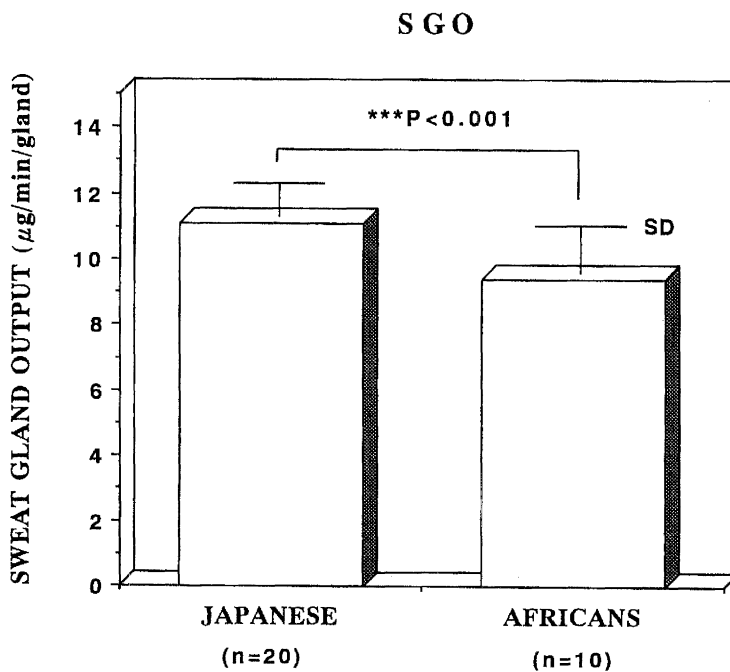
S G O

Fig. 6. Comparison of sweat gland output per single gland activated by ACh iontophoresis between the Japanese and the Africans.

DISCUSSION

Onset–time, AXR and DIR sweating in QSART

Tropical inhabitants possess heat–tolerance due to enhanced dry heat loss such as radiation, convection and conduction and due to an avoidance of excess sweat loss (non–evaporating, ineffective sweating), which is convenient to maintain body fluid and osmolarity (Matsumoto et al, 1997).

QSART clearly showed a longer onset–time and the smaller AXR and DIR responses in the Africans compared to the Japanese in this study. These results support our earlier findings that suppressed sudomotor function in tropical natives is attributed not only to the suppression of central sudomotor mechanism (upward threshold shift for sweating) but also to the suppressed sweat gland sensitivity to ACh (Matsumoto et al, 1997). However, these observations are not in agreement with other reports which suggested that reduced sweating in tropical natives is attributed to habituation of central thermoregulatory mechanisms to heat, and the secretory capacity of the sweat gland per se has been enhanced by long–term heat acclimatization (Kuno, 1956; Ogawa and Sugenoya, 1993). Chen and Elizondo (1974) showed the depression of thermal sweating after repeated ACh iontophoresis. It is suggested that sweat glands of tropical subjects were desensitized by repeated exposure to ACh secreted from sudomotor nerve terminals through long–term residence in tropics. Subthreshold sudomotor neural impulses originate in central nervous system in the absence of visible sweating (Ogawa and Bullard, 1972). The lower QSART responses in tropical subjects may be attributed to the lower central sudomotor efferent signal due to the lower oral temperature. The local skin temperature is an important factor to modulate sweating response not only as an input to central regulatory mechanism, but as a local effect on activity of sweat glands. Local heating facilitates transmitter release at the neuroglandular junction and also augments glandular responsiveness to the transmitter (Ogawa and Asayama, 1986). The DIR response increased 1.5% and the AXR increased 3%, when the skin temperature elevates by 1℃ (Low et al, 1983). The difference of skin temperature of 0.3℃ never explain the 72–110% of QSART differences between the African and the Japanese in this study.

Sweat gland density and sweat gland output

Total number of active sweat glands remains unchanged throughout life beyond the age of about 2.5 years. The number in the Philippines is larger than the Japanese (Kuno, 1953). Therefore, the total number of active sweat gland is thought to be dependent on the climate where the man lived during such early life. Sweat gland density decreases (Bar–Or, 1980) and sweat gland output increases (Matsumoto et al, unpublished data) as the BSA increases with the growth. The sweat gland density was 50.6% higher and the sweat gland output per single gland was 20.4% larger in the Japanese compared to the Africans. These results indicate that ACh sensitivity of the sweat glands in tropical subjects is suppressed at both the points of sweat gland recruitment and of single gland output, compared to temperate Japanese subjects. These finding are not in agreement with the earlier reports (Kuno, 1953; Ojikutu,

1965). Sweat gland density observed in this study reveals the number of sweat glands activated by ACh. According to Low et al. (1992), QSART with 10% of ACh and 2 mA of direct current provides nearly maximal sweat gland stimulation. However, if ACh sensitivity of the sweat glands is suppressed, QSART might not provide maximum stimulation in tropical subjects. It is still possible that the maximum sweating capacity of tropical subjects is larger than temperate subjects. Further studies are expected to clarify the mechanisms of long–term heat acclmatization in human beings.

Oral temperature and skin temperature in tropical natives

In this study, resting oral temperature in the Africans were slightly but significantly lower than the Japanese under thermoneutrality. Tropical subjects were studied just after arrival to Japan during cold winter season, in order to minimize the influence of acclimation to cold in Japan. Core temperature measurement could be influenced by the experimental conditions such as the device, site and protocol of measurements and environmental conditions. Whether the resting core temperatures in tropical natives should be high or low is controversial. Higher core temperature in tropical natives has been reported in the earlier papers as the term of climatic fever (Renbourn, 1946). However, in those papers, methods and conditions of core temperature measurements were not well controlled. Hori, et al. (1977) studied basal metabolic rate and oral and skin temperatures in 30 Thai and 20 Japanese and reported that the oral temperature and basal metabolism were identical. We have also reported identical oral temperatures for Thai and Japanese (Matsumoto et al, 1991, 1993). The lower skin temperature on the extremities in tropical natives is in agreement with the report by Hori et al. (1977).

Rise in skin temperature due to ACh iontophoresis

Vasodilatation induced by ACh applied iontophoretically to the skin has been reported (Morris and Shore, 1996). Furthermore, cutaneous vasodilatation responses synchronize with sweat expulsions of natural sweating (Sugenoya et al, 1995). We also observed elevation of skin temperature accompanied with sweating (Matsumoto et al, unpublished data). In this study, local skin temperature elevated with sweat onset during ACh iontophoresis. These results strongly support the idea that skin vasodilatation is accompanied with sudomotor activities. In conclusion, the fewer activated sweat glands and the lesser sweat output per single gland by iontophoretically applied ACh were observed in the Africans. These results indicate that suppressed thermal sweating in Africans is, at least in part, attributed to the suppressed glandular sensitivity to ACh through both recruitment of sweat glands and sweat output per single gland.

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