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Spermidine and Spermine Are Enriched in Whole Blood of Nona/Centenarians

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Abstract

Polyamines (putrescine, spermidine, and spermine) are a family of molecules that derive from ornithine through a decarboxylation process. They are essential for cell growth and proliferation, stabilization of negative charges of DNA, RNA transcription, translation, and apoptosis. Recently, it has been demonstrated that exogenously administered spermidine promotes longevity in yeasts, flies, worms, and human cultured immune cells. Here, using a cross-sectional observational study, we determined whole-blood polyamines levels from 78 sex-matched unrelated individuals divided into three age groups: Group 1 (31–56 years, $n=26$, mean age 44.6 ± 6.07), group 2 (60–80 years, $n=26$, mean age 68.7 ± 6.07), and group 3 (90–106 years, $n=26$, mean age 96.5 ± 4.59). The total content of polyamines is significantly lower in groups 2 and 3 compared to group 1 ($p=3.6\times10^{-12}$). Interestingly, this reduction is mainly attributable to the lower putrescine content. Group 2 displays the lowest levels of spermidine and spermine. On the other hand, nona/centenarians (group 3) display a significantly higher median relative percentage content of spermine with respect to total polyamines, compared to the other groups (13.2% vs. 14.1% vs. 30.6%, $p=6.0\times10^{-4}$). For the first time, we report profiles of polyamines from the whole blood of healthy nona/centenarians, and our results confirm and extend previous findings on the role of polyamines in determining human longevity. However, although we found an important correlation between polyamines levels and age groups, further studies are warranted to fully understand the role of polyamines in determining life span. Also, longitudinal and nutritional studies might suggest potential therapeutic approaches to sustain healthy aging and to increase human life span.

Introduction

The aliphatic polyamines putrescine, spermidine, and spermine are normal cell constituents that play important roles in cell proliferation and differentiation. The equilibrium between cellular uptake and release and the balanced activities of biosynthetic and catabolic enzymes of polyamines are essential for normal homeostasis in the proliferation and functions of cells and tissues (protein synthesis, transcription, apoptosis). However, it was reported that certain types of cancer are incident to elevated putrescine and cadaverine concentration in tissue, *e.g.*, putrescine accumulates in human pulmonary tumors, blood, serum, and mucus of cancer patients.[1] On the other hand, previous studies have demonstrated that spermine and spermidine suppress inflammatory mediators, such as proinflammatory cytokines, both *in vitro* and *in vivo*.[2–4]

Nevertheless, the role of polyamines in human longevity and in age-related diseases has been underscored. Recently, it has been reported that exogenous levels of spermidine induce autophagy, which in turn increases life span in several model organisms, from yeast to mice.[5,6] Interestingly, Δ*spe1* yeast cells (deficient in the ornithine decarboxylase SPE1, which catalyzes the first step of polyamine biosynthesis) exhibited increased mortality, which could be restored to normal levels by supplementation with low doses (0.1 mM) of spermidine or its precursor putrescine.[5] Thus, both chronological aging (which constitutes a model of postmitotic aging) and replicative aging (which constitutes a model of stem cell aging) of yeast cells were significantly inhibited by spermidine supplementation. Furthermore, it has also been demonstrated that spermidine administration potently inhibited oxidative stress in aging mice.[5,6]

Polyamine levels decrease with age in many organisms, including humans.[7] A negative correlation has been reported between age and both spermidine and spermine content in different areas of human brains, evidence of an involvement of these polyamines in white matter modifications during

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aging.[7,8] The polyamine levels in serum and urine of healthy human donors are also age-related, diminishing progressively with increasing age.[9] By analyzing the age dependence of polyamines metabolic enzymes and polyamines occurrence in cancer tissues, Scalabrino and Ferioli have collected data showing the impact of reduced polyamine biosynthetic capacity in aged mammals in delaying the course of some tumors in elderly patients.[10] Owing to their multiplicity of actions, the interaction between polyamine levels, aging, and disease is very complex to disclose. Although spermidine supplementation has been found to ameliorate oxidative stress resistance in some model organisms, high levels of polyamines are associated with many diseases, including cancers. One main reason of the controversial outcomes concerning polyamines has been proposed to arise from the tendency to consider these different molecules as a unique family with common properties.[7] On the contrary it has been described that different polyamines can have different roles, despite their very similar structure. Concerning aging, besides the beneficial role of moderate spermidine supplementation against age-related diseases,[6] rather few data have been reported about the distribution of endogenous polyamines during healthy aging in humans and the relative balance between them to contribute positively to the health of the organism.

Taking into account the significant impact of polyamines on life expectancy (spermidine in particular), here we quantified the total polyamine content in whole blood of long-lived, aged, and young/adult healthy donors, respectively, and also evaluated the relative distribution of putrescine, spermidine, and spermine.

Materials and Methods

Subjects

Peripheral whole blood was obtained from 78 (39 females and 39 males) unrelated individuals with Caucasian ancestry, free of clinically overt pathologies (*e.g.*, cancer, diabetes, heart diseases, hypertension, obesity, and chronic inflammatory diseases) and randomly recruited from the same geographical area of central Italy. Subjects were recruited in blood donor centers and through family physicians. The study population was divided into three age groups: Group 1 (31–56 years, $n=26$, 13 males and 13 females, mean age 44.6 ± 6.07), group 2 (60–80 years, $n=26$, 13 males and 13 females, mean age 68.7 ± 6.07), and group 3 (90–106 years, $n=26$, 13 males and 13 females, mean age 96.5 ± 4.59). A total of 3 mL of whole blood was collected from each subject during fasting, in a K_2EDTA-containing vacutainer.

The study protocol was approved by the Joint Ethical Committee (JEC) University of Camerino-Azienda ASUR Marche ZT-10 Camerino, in accordance with the Declaration of Helsinki in its revised edition and with international and local regulatory requirements.

Precolumn derivatization and high-performance liquid chromatography separation of polyamines

Direct determination of polyamines in biological fluids is difficult because they are small aliphatic molecules that do not exhibit any structural features that would allow their sensitive spectral detection without derivatization. Thus, 100 μL of whole blood (previously treated with 5% trichloroacetic acid (TCA) to allow protein precipitation) and 1 mL of standard polyamines stock solutions (1 mg/mL) were subjected to a derivatization process with dansyl chloride according to Seiler.[11] After incubation at 40°C for 45 min with dansyl chloride, mixtures were filtered through a 0.45-μm regenerated cellulose-membrane (ALBET-LabScience, Dassel/Relliehausen, Germany) and injected into a high-performance liquid chromatography (HPLC) apparatus (Agilent 1100) for analysis. The separation was executed on a Supelcosil LC 18 RP column (5 μm; 4.6×250 mm) maintained at 35°C. The mobile phase was composed of water and methanol with the gradient elution system at a flow rate of 1.0 mL/min. The initial mobile phase contained 70% methanol for 7 min. The gradient volume of methanol was 70%–75% at 7–10 min, 75%–90% at 10–20 min, and 90%–100% at 20–25 min.[12] The signal detection was through a diode array detector (DAD), and the chromatograms were recorded at a wavelength of 253 nm. Polyamine calibration curves were obtained using dansylated polyamine standard solutions (concentrations ranging between 0.001 mg/mL and 0.1 mg/mL).

A reference sample (nondansylated blood sample) was subtracted from each dansylated blood sample analyzed to remove interference from substances present in blood eventually absorbing at the same wavelength of dansylated polyamines. Polyamine concentration was normalized to total protein concentration, obtained with Bradford assay, and was expressed as nmol/mg.

Statistical analysis

Statistical analyses were performed by using the SPSS 18.0 software (SPSS, Chicago, IL). Because quantitative data did not follow normal distributions (by the Kolmogorov–Smirnov test), the differences between the three age groups were evaluated by the nonparametric Kruskal–Wallis test. Two-tailed p values are given throughout. Statistical significance was set at $p<0.05$.

Results

The proposed HPLC method was applied to determine the putrescine, spermidine, and spermine concentrations in the whole blood samples of the three age groups under study. The derivative polyamines were identified from the comparison of retention times characteristic of standard polyamines (see Figure S1A)(Supplementary Data are available at www.liebertonline/rej/), to that obtained from whole blood samples (Fig. S1B). The relative content was quantified according to the linear regression obtained from each calibration curve (Fig. S2).

The detected polyamines total content is reported in Fig. 1. According to previous evidence,[10] we noticed a significant reduction for total polyamines content in groups 2 and 3 compared to group 1 (Kruskal–Wallis $H=26.42$, degrees of freedom [df] 2, $p=1.8\times10^{-6}$). Notably, the lower total polyamines content detected in groups 2 and 3 was mainly attributable to the lower putrescine level compared to age group 1 (data not shown). In fact, groups 2 and 3 displayed negligible levels of putrescine (<0.013 nmol/mg) for the majority of subjects studied (26/26 for group 2 and 18/26 for group 3, respectively), whereas 20/26 of subjects in group 1 showed relevant levels of putrescine (0.149–0.441 nmol/mg).

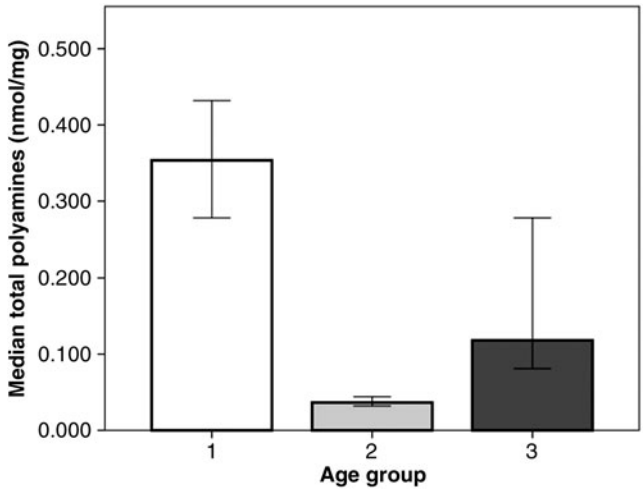

FIG. 1. Polyamines total content (expressed as nmol/mg) across the three age groups. Values are expressed as median ±95% confidence interval. The differences between the three age groups were evaluated by a nonparametric Kruskal–Wallis test ($p = 3.6 \times 10^{-12}$).

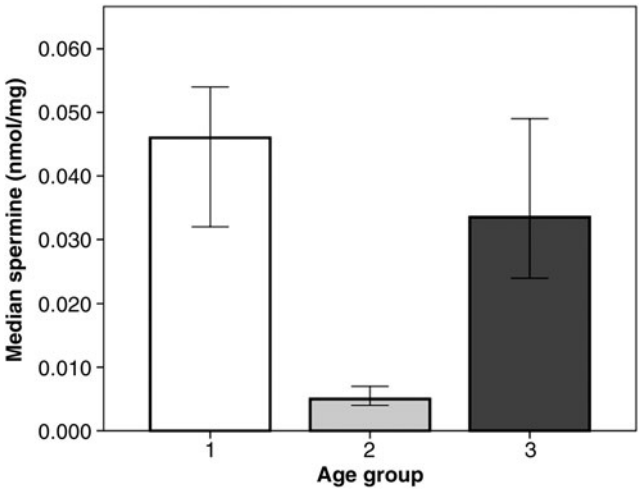

FIG. 3. Spermine levels (expressed as nmol/mg) across the three age groups. Values are expressed as median ± 95% confidence interval. The differences between the three age groups were evaluated by a nonparametric Kruskal–Wallis test ($p = 4.7 \times 10^{-12}$).

Spermidine levels were significantly different across the three age groups (Fig. 2), with group 3 displaying levels similar to group 1, whereas group 2 subjects showed lower levels compared to the other two groups (Kruskal–Wallis H = 44.19, df 2, $p = 2.5 \times 10^{-10}$). A similar result was obtained for spermine (Fig. 3), with group 2 displaying the lowest levels (Kruskal–Wallis H = 52.16, df 2, $p = 4.7 \times 10^{-12}$). Importantly, when expressing polyamines content as the relative percentage with respect to total polyamines in each sample, group 1 showed the lowest median relative percentage content of spermidine compared to the other groups (16.6% vs. 65.4% vs. 61.9%, Kruskal–Wallis H = 23.33, df 2, $p = 1.4 \times 10^{-5}$) (Fig. 4). Moreover, group 3 reported the highest median relative percentage content of spermine (13.2% vs. 14.1% vs. 30.6%, Kruskal–Wallis H = 15.01, df 2, $p = 6.0 \times 10^{-4}$) (Fig. 5). Because females are known to have a longer life expectancy, we also analyzed the data according to gender, but no significant difference compared to pooled data analyses was noticed (data not shown).

Discussion

To our knowledge, this is the first study on whole blood polyamine levels in reference to human longevity. Recent evidence points to an important role of polyamines in determining longevity. In particular, spermidine has been assumed to be a new longevity drug that can increase life span in yeast, nematodes, flies, and mice, possibly through an

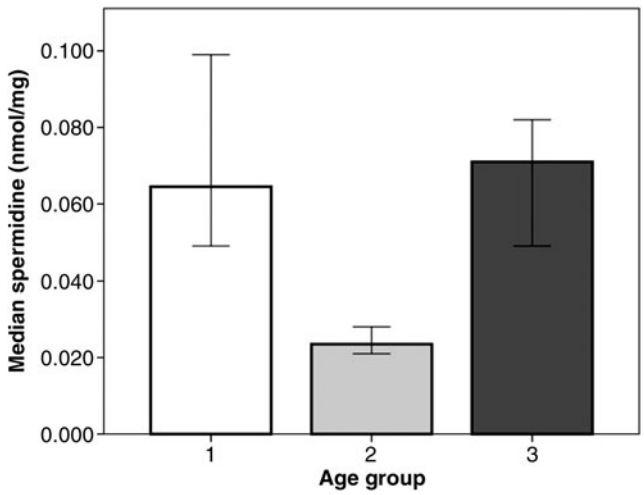

FIG. 2. Spermidine levels (expressed as nmol/mg) across the three age groups. Values are expressed as median ± 95% confidence interval. The differences between the three age groups were evaluated by a nonparametric Kruskal–Wallis test ($p = 2.5 \times 10^{-10}$).

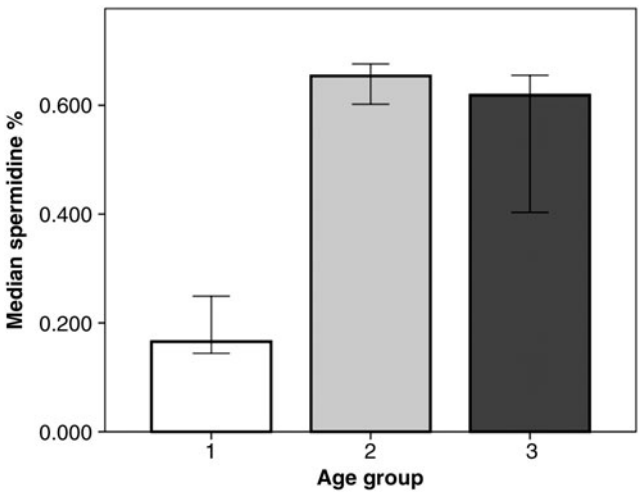

FIG. 4. Median values of relative percentage of spermidine in whole blood from the three age groups. Values are expressed as median ± 95% confidence interval. The differences between the three age groups were evaluated by a nonparametric Kruskal–Wallis test ($p = 1.4 \times 10^{-5}$).

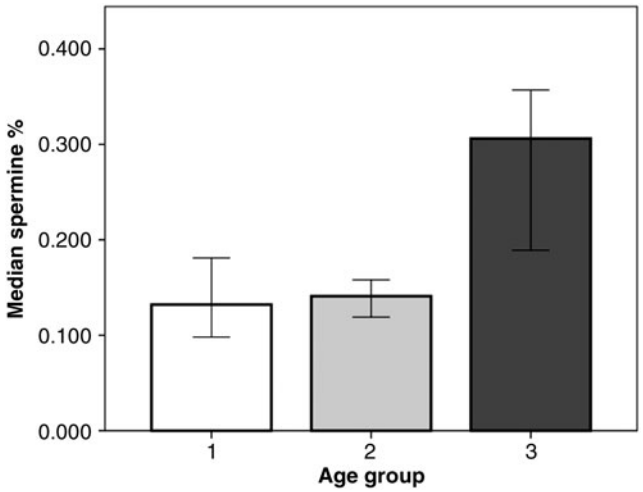

FIG. 5. Median values of relative percentage of spermine in whole blood from the three age groups. Values are expressed as median ± 95% confidence interval. The differences between the three age groups were evaluated by a nonparametric Kruskal–Wallis test ($p = 6.0 \times 10^{-4}$).

effect on chromatin-mediated regulation of gene expression and by mediating autophagy processes.[5] Moreover, because spermidine is a natural component of our diet, and several foods are known to be rich in spermidine, including soy beans, tea leaf, and mushrooms, a therapeutic use has been also suggested.[13]

The present work has been focused on age-related polyamine profiling in whole blood, including both endogenous intracellular content of blood cells and circulating polyamines, in part absorbed through the diet. Our data are consistent with already published data obtained by HPLC determination in whole blood from human volunteers.[14] In their work, Soda et al. reported that a long-term oral intake of polyamine-rich diet causes an increase in blood polyamine levels (especially in spermine concentration). On the contrary, they demonstrated that a short-term supplementation of polyamine-rich food does not increase blood polyamine levels. This observation allows us to consider our data likely unaffected by the wide variability of polyamine content among foods, and in any case randomly affected. On the other hand, we are aware that polyamine concentrations in the blood can vary considerably among healthy individuals, as observed by others.[3,15,16] Several factors can contribute to this variability; polyamines produced in the intestinal lumen are quickly absorbed by blood because of the highly microvascularized intestinal mucosa, and gut microbiota are also an efficient source of polyamines.[17,18]

Here we found that age-related decrease in polyamine levels is attributable to a very significant reduction in putrescine content, whereas spermidine and spermine are significantly enriched in whole blood of nona/centenarians. Interestingly, high putrescine levels were reported in several age-related diseases such as cancer,[1,10] Parkinson disease,[19] pancreatitis,[20] and ischemia.[21] Notably, spermidine and spermine concentrations were markedly decreased in Alzheimer disease.[22] On the other hand, spermidine and spermine were found to ameliorate some pathological conditions. Spermidine supplementation improves protein utilization efficiency and ameliorates trauma effects on amino acid levels[23]; it has also been suggested that oral spermidine administration could be a useful treatment for multiple sclerosis.[24] Spermine confers protection against lethal sepsis partly by attenuating sepsis- and high-mobility group protein B1 (HMGB1)-induced inflammatory responses,[25] and it

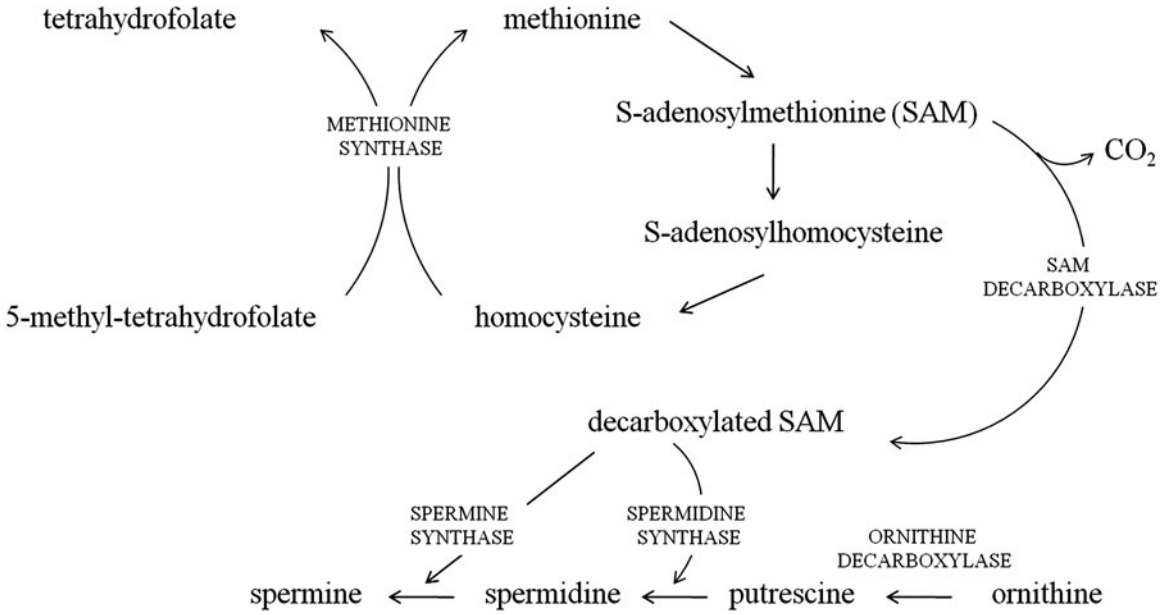

FIG. 6. Methionine cycle and polyamine pathway. Spermidine and spermine biosynthesis is dependent on *S*-adenosylmethionine (SAM) levels. Methionine is regenerated from homocysteine in a folate-dependent trans-methylation reaction leading to subsequent formation of SAM, which is converted to decarboxylated SAM by SAM decarboxylase. Spermidine is synthesized from putrescine, and spermine from spermidine, by transfer of the aminopropyl moiety of decarboxylated SAM.

has been proven to reduce infarction and neurological deficit following middle cerebral artery occlusion.[26]

The underlying mechanisms of age-related alterations in the relative content of whole blood polyamines observed in this study are not fully understood at present. Given the observational nature of this study, it is very difficult to explain whether the changes in the polyamines levels between the three age groups are due to a decrease in cellular biosynthesis of polyamines (*e.g.*, reduced cellular mitotic rate) or due to a reduction in metabolic uptake from the diet.

Nevertheless, our data add new insight about the relevance of polyamines in human longevity. A distinct role of the three physiological polyamines has been proposed to regulate the different cellular functions in which this class of molecule is involved.[27] Cell proliferation and apoptosis are strictly related to polyamine intracellular levels, which are regulated by multiple mechanisms affecting their synthesis, degradation, uptake, and excretion. The enzymes involved in these biochemical processes are finely regulated; thus, an increase of one of the three natural polyamines must have a biological meaning. Many investigations seem to converge to the observation that rapidly growing prokaryotes mainly contain putrescine and spermidine, whereas slowly growing eukaryotes mainly contain spermidine and spermine.[27] The relationship between polyamine content and rate of cell proliferation is also supported by the hypothesis that the reduced polyamine biosynthetic capacity of aged mammals can account for the slower course of some tumors in elderly patients.[10] Aging is a biological phenomenon significantly influenced by both genetic and environmental factors.

Gene-related regulatory systems (evolutionarily conserved) also control diapause-like states allowing the postponed reproduction in response to adverse environmental conditions.[28] Spermidine has been shown to act synergistically with resveratrol (a natural polyphenol found in grapes, red wine, berries, knotweed, peanuts, and other plants, and a known sirtuin 1 [SIRT1] activator) in inducing autophagy through convergent deacetylation and acetylation reactions in the cytosol and in the nucleus.[29] Polyamines (spermidine, in particular) could modulate the cell cycle in response to environmental changes (*i.e.*, oxidative stress, food limitation, and crowding), induce autophagy, and eventually increase life span.[30]

Notably, methionine cycle and polyamine pathway are interdependent (Fig. 6),[27] and high homocysteine levels (marker of folate deficiency) have been associated with cardiovascular disorders.[31] Recently, a surprisingly higher content of plasmatic homocysteine in centenarians compared to randomly selected donors has been reported.[32] The authors suggest the existence of a protective mechanism (probably gene related), allowing a long survival despite the high value of homocysteinemia. Cellular resistance to homocysteine, as a key mechanism for successful aging, has been also proposed by others.[33] In this scenario, the relative increase of spermidine in the blood of nona/centenarians we observed might be viewed in contrast with high levels of homocysteine, unless compensatory mechanisms related to vitamin intake (*e.g.*, folate) or to the enzymatic activities of polyamine pathway (in particular ornithine decarboxylase [ODC] and *S*-adenosylmethionine decarboxylase) are implied.[34]

Clearly, the present work should be considered a preliminary investigation, and further studies are warranted to fully understand the role of polyamines in determining life span. Also, longitudinal and nutritional studies might suggest potential therapeutic approaches to sustain healthy aging and to increase human life span. In conclusion, our study extends our knowledge on biochemical determinants of human longevity and confirms the relevance of polyamines in biological processes.

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Author Disclosure Statement

No competing financial interests exist.

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