— **Review** —

Early Development Origins of Adult Disease Caused by Malnutrition and Environmental Chemical Substances

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We observed that maternal exposure to diesel exhaust (DE) and diesel exhaust particles (DEPs) damaged the reproductive and central nervous systems in mice and rats. These observations suggest that impairment of early development induced by maternal exposure to DE and DEP causes several disorders after growing up. To elucidate the effects of maternal exposure to environmental substances, we review here a hypothesis of fetal and early developmental origins of adult disease. Recent studies influenced by Dr. Barker's Thrifty Phenotype Hypothesis have led to advances in understanding how fetal and infant malnutrition can permanently and adversely alter the development of tissues and organs. Several epidemiological surveys in humans have uncovered links between maternal malnutrition and effects on the organs such as the kidney, pancreas, liver, muscles, adipocytes, and the hypothalamic-pituitary-adrenal (HPA) axis. These observations were examples of critical period programming. The idea has been applied to examining possible fetal and early origins of other diseases. Interestingly, many reports showed that similar phenomena were induced by perinatal exposure to airborne environmental pollutants. Studies have shown that maternal DE exposure disrupts reproductive development and damages the central nervous system. In addition, perinatal exposure to tobacco smoke has been linked to several respiratory disorders. These results show that early development is a critical determinant of adult physiology and much care should be taken to ensure the proper environment for fetal development. This idea is especially topical currently, where rapid industrialization in Asia has accelerated changes in environment and increased pollution.

Key words —— thrifty phenotype hypothesis, early development, maternal exposure, diesel exhaust, environmental tobacco smoke, critical period programming

INTRODUCTION

We observed that mice that were maternally exposed to diesel exhaust (DE) and diesel exhaust particles (DEPs) showed signs of damage to the reproductive and central nervous systems. These observations suggest that impairment of early development induced by maternal exposure to DE and DEP causes several disorders after growing up. To elucidate the effects of maternal exposure to environmental substances, we review here a hypothesis of fetal origins of adult disease and its related references.

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The main theory of fetal origins of adult disease was put forth by Dr. David J.P. Barker in the early 1990's.[1, 2] The hypothesis stated that physiological development *in utero* is tailored to the environment that the fetus indirectly senses through the mother. Then, development of certain organs ceases either *in utero* or postnatally and certain features become permanent. If the environment after birth is different from the one sensed by the fetus, these permanent changes can be maladaptive and lead to adult disease. The specific example that Dr. Barker considered is the link between perinatal malnutrition and offspring adult diseases related to metabolic syndrome. He theorized that some cases of adult disease can be attributed to an adverse environment (*e.g.*, malnutrition) during fetal development. This malnutrition then leads to permanent changes in the growth, metabolism, and vasculature of various organs which predisposes the child to adult disease.

Since publication, Dr. Barker's hypothesis has gained much attention in the scientific community and has even garnered the interest of the popular press.[3,4] His ideas are particularly applicable to the present, where countries such as China and India are rapidly industrializing, with several areas that transitioning from impoverished to relatively affluent within the current generation. A recent epidemiological study in South India has already noted the effects of such rapid changes in environment on the prevalence of adult coronary heart disease.[5]

In addition to this background theory, we also take a brief look at the effects of perinatal environmental tobacco smoke (ETS) exposure on respiratory system development and review experiments conducted by our laboratory on the effects of maternal DE exposure on the reproductive and central nervous systems.

EPIDEMIOLOGICAL EVIDENCE SUPPORTING A LINK BETWEEN MATERNAL MALNUTRITION AND ADULT DISEASE

The earliest origins of Dr. Barker's hypothesis came from epidemiological studies relating adult coronary heart disease and measurements taken at birth, specifically birth weight[6] and ponderal index,[7] a measure of thinness defined as the birthweight divided by the cube of the crown-to toes length at birth (Fig. 1A). These simple studies showed that babies born with lower birth weight or lower ponderal index were more likely to develop coronary heart disease in later life.[8] Since it is known that fetal development is at least limited in part by nutrient supply in the womb, many cases of thinness at birth are indicative of earlier malnutrition. Thus, the above evidence indicates a link between fetal malnutrition and adult disease. Other studies looking at birth weight and ponderal index at birth have also linked these parameters to hypertension[9] and type 2 diabetes.[10] Another important piece of evidence came from a longitudinal study of adult coronary heart disease in males in Helsinki that examined hazard ratios for adult coronary heart disease versus the ponderal index at birth and body mass index (BMI) at 11 years old.[11,12] The data showed that boys who were born thin but grew and reached an average BMI at age 11 had higher risk for adult coronary heart disease (this asymmetric growth pattern is called catch-up growth), whereas boys who were born with normal ponderal index had lower risk even if they reached an above average BMI at age 11 (Fig. 1B). This evidence suggests that the thinness at birth, possibly caused by maternal malnutrition, led to permanent changes in development that could not be recovered through later growth.

This data also illustrates another important aspect of the hypothesis. Changes in prenatal development are not disadvantageous in themselves; the boys who were born thin but continued to have a low BMI at age 11 had normal or low risk for adult heart disease. However, boys who were born thin

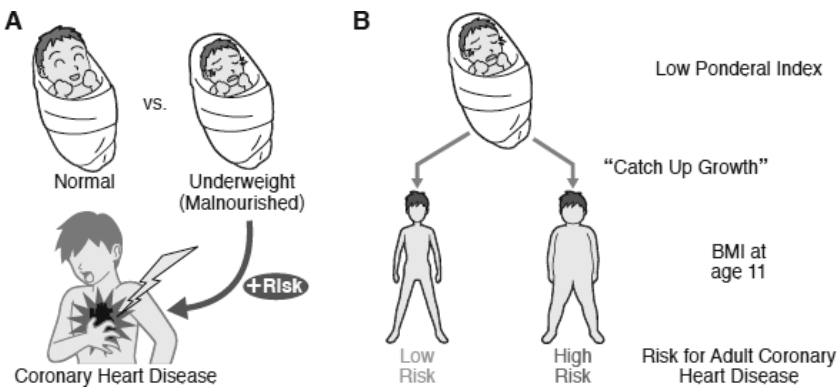

Fig. 1. Hazard Ratios for Coronary Heart Disease Have Inverse Correlation to Ponderal Index and BMI at 11 years

(A) Several epidemiological studies showing a negative relationship between birthweight[6] and ponderal index[7] and adult coronary heart disease risk led Dr. Barker to formulate his hypothesis that some cases of adult heart disease have origins in fetal malnutrition. (B) Another epidemiological study[11,12] showed that babies that had the highest risk for adult coronary heart disease were those that were born thin (low ponderal index) but then achieved above average BMI at age 11. Babies that were born thin and reached low BMI at age 11 and babies that were born with average ponderal index and reached average ponderal index both had low risk for coronary heart disease. This evidence suggests that it is the change in the growth, probably due changes in availability of nutrients, that creates the risk for adult disease.

but then experienced catch-up growth had greater risk for adult heart disease. This suggests that it is the change in environment, specifically the increase in growth, after birth that is important.

EFFECTS OF MATERNAL MALNUTRITION: THE THRIFTY PHENOTYPE HYPOTHESIS

As the theory gained acceptance and corroborating evidence from other similar epidemiological studies,[13, 14] much research into the exact mechanism behind the changes in fetal development and their ramifications on adult life has been conducted. In 2001, Drs. Barker and C. Nicholas Hales put forth and updated form of the theory[15] which diagrams several key organs affected by maternal malnutrition. The proposed developmental pathways that fetal environment acts on were based on both epidemiological studies as well as preliminary experimental studies in animal models. This report will concentrate on the four targets that Drs. Barker and Hales considered to be critical in the programming of adult disease: kidney; pancreas; muscle, liver, and adipose tissue; and hypothalamic-pituitary-adrenal (HPA) axis.

Effect of Maternal Malnutrition on the Kidney

Maternal malnutrition is hypothesized to cause changes in the kidney which lead to adult hypertension and renal failure. There is epidemiological evidence linking fetal malnutrition to hypertension in humans.[2] In addition, research in animal models has led to the development of an initial hypothesis of the mechanism.

Studies in rats and sheep have shown that maternal malnutrition leads to a decrease in the amount of nephrons in the adult offspring.[16] In addition, human offspring that experienced intra-uterine growth restriction (IUGR), indicative of fetal malnutrition, also had decreased nephron number in adulthood (Fig. 2).[16] This decreased nephron number could be due to selective shunting of blood and precious nutrients away from the kidney to more critical organs, such as the brain, in response to maternal malnutrition because of the lower excretory demand of an underweight baby. This concurs with data from autopsy studies indicating that birthweight is a good predictor of nephron number in children ages 1–18.[17] Since nephrogenesis stops after birth,[18] this decreased nephron number is permanent. As the child grows, the nephrons must enlarge in size to cope with the increased excretory demand.

Taking into account the fact that only babies that exhibited catch-up growth had greater risk for coronary heart disease, researchers have developed a tentative hypothesis explaining the effect of lower nephron number and catch-up growth on adult disease.[19] The asymmetric catch-up growth is hypothesized to increase adult excretory load on babies who experience catch-up growth after fetal malnutrition because the number of nephrons is unable to keep up with the increased excretory demand following the accelerated growth after birth.

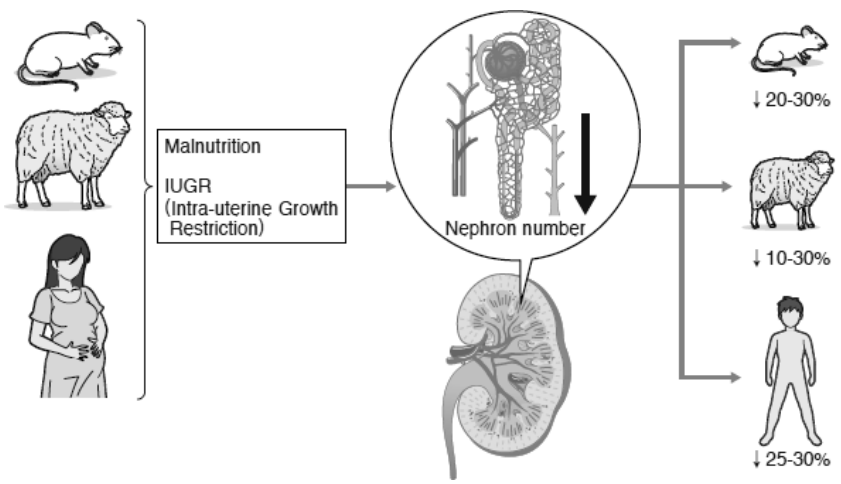

Fig. 2. Maternal Malnutrition and Low Nephron Number: Diagrammatic Representation

Studies in rats and sheep have shown that maternal malnutrition leads to decreased nephron number in the adult offspring.[16] In addition, studies of human intra-uterine growth restriction, indicative of maternal malnutrition, show that this also leads to lower offspring nephron number.[16]

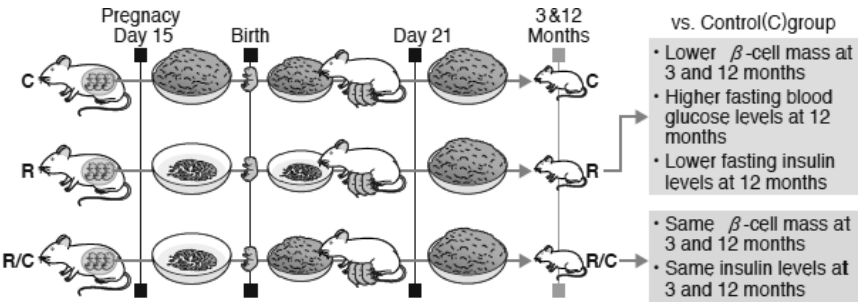

Fig. 3. Effect of Aging on β-cell Mass and Function in Rats Malnourished during the Perinatal Period

Garofano *et al.*[22] studied three groups of mice Control (C), Food restricted (R), and a hybrid group (R/C). From pregnancy day 15 until birth, the C mothers were fed *ad libitum* while the R and R/C mothers were fed a 50% diet. After birth and until weaning on day 21, the C and R mothers nursed their own offspring and were fed the same diets as during the pregnancy period. However, the R/C offspring were nursed by control mothers. After weaning, all offspring were fed *ad libitum* until 3 and 12 months, when data was collected. The researchers discovered that, compared to the C group, the R group had lower β-cell mass at 3 and 12 months as well as higher fasting blood glucose levels and lower insulin levels at 12 months. The R/C group, in contrast, had the same β-cell mass and insulin levels at both time points.

Temporary excretory overload is known to cause afferent dilation and efferent constriction in glomeruli, which increases the glomerular capillary pressure. Persistently high glomerular capillary pressure is associated with higher risk of renal failure due to the increased load on each nephron.[20] In addition, it is known that the excretory overload causes hypertrophy of the vessels in the nephron. Vallon *et al.*[21] have put forth a hypothesis related to diabetes that is believed to be relevant to hypertension as well.[16] They believe that the vessel hypertrophy following excretory overload mainly leads to proximal tubule enlargement and elongation, thus decreasing the amount of sodium ion delivered to the macula densa and causing activation of the renin-angiotensin system, which is associated with hypertension.

Effect on the Pancreas

Garofano *et al.*[22] have shown that 3-month-old rats whose mothers were fed an isocaloric low-protein diet during pregnancy and lactation have a reduced β-cell mass and a corresponding reduction in insulin response to glucose challenge (Fig. 3). However, the glycaemic response remained unchanged, possibly due to increased insulin sensitivity.[22] It is known that aging in humans leads to an increase in fasting and post-challenge glucose levels despite similar insulin response levels. This is consistent with the results for the control group of rats at 12 months of age. The experimental group continued to have decreased insulin response at 12 months and had higher fasting blood glucose levels.

The researchers noticed that at 3 months, β-cells from malnourished rats had higher rates of apoptosis. Since it has been previously shown that a wave of β-cell apoptosis shortly before weaning remodels the pancreas,[23,24] they hypothesized that the malnourished rats undergo a wave of β-cell apoptosis to get rid of a large number of β-cells either damaged or not needed after weaning. Initially, the effects of this remodeling of the pancreas on glucose metabolic function may be counteracted by the increased insulin sensitivity. However, as the effect of aging sets in, it appears that the earlier remodeling causes higher blood glucose levels.

Effect on Muscle, Liver, and Adipose Tissue

The third pathway involves insulin resistance programming in the muscle, liver, and adipose tissue. Studies using the low protein rat model (where maternal mice are fed an isocaloric low protein diet until weaning[25]) have shown that these tissues in malnourished rats display equal, if not better glucose tolerance at 3 months, probably due to changes in insulin receptor levels.[26] However, after aging, the malnourished rats had the same levels of insulin receptors as the controls and displayed lower glucose tolerance.

Liver tissue samples of 3-month-old perinatally malnourished rats have an 80% reduction in expression of glucagon receptors and upregulation of insulin receptors.[27] In addition, these livers were observed to undergo physical changes such as enlargement of lobules.[28] Muscle strips of 3-month-old perinatally malnourished rats also have increased expression of insulin receptors, which may explain their higher insulin sensitivity.[29] However, by 15 months of age, this same group of rats show lower insulin sensitivity and the num-

ber of receptors has become similar to the control group.[30] Finally, adipocytes of 3-month-old perinatally malnourished rats have higher basal and insulin-stimulated glucose uptake probably due in part to greater insulin receptor expression.[31] However, at 15 months, the adipocytes are resistant to the stimulatory and antilipolytic actions of insulin.[32] These age-dependent glucose challenge results are similar to what was observed by Garofano *et al.*[22] Since insulin resistance is only observed after the level of insulin receptors dropped, the molecular defect appears to lie downstream of receptor itself.

Effect on the Hypothalamic-Pituitary-Adrenal Axis

The final pathway involves the HPA axis. Studies have shown that maternal malnutrition leads to down regulation of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2),[33] which is an enzyme that catalyzes metabolism of maternal cortisol and corticosteroid into inert products and is found in very high levels in the feto-placental barrier.[34] It breaks down 80–90% of the active maternal glucocorticoids and thus serves as a potent barrier protecting the fetus from glucocorticoids. Downregulation of 11β-HSD2 is hypothesized to allow more active maternal glucocorticoids to pass through this barrier reach the fetus. The hypothesis is supported by studies that show that maternal malnutrition causes abnormal adult HPA function in rats[35] and sheep.[36] Studies in rats have also shown that prenatal exposure to glucocorticoids permanently increases glucocorticoid releasing hormone mRNA levels in adults.[37,38] Finally, elevated glucocorticoid levels in adults are known to be risk factors for hypertension and, in rat models, have been implicated in adult glucose intolerance.[39]

CRITICAL PERIOD PROGRAMMING

The thrifty phenotype hypothesis is an example of critical period programming, a term that has been gaining more and more popularly recently. Dr. Barker explains it as "a critical period when a system is plastic and sensitive to the environment, followed by loss of plasticity and a fixed functional capacity."[8] The idea has been applied to examining possible fetal and early origins of other diseases. In particular, there have been several studies looking at the effects of perinatal exposure to airborne environmental pollutants. Two of the most commonly occurring and potent sources of airborne particles are DE and ETS. The remainder of this report will be devoted to looking at the effects on early development of exposure to these two particulate pollutants.

EFFECTS OF MATERNAL EXPOSURE TO DIESEL EXHAUST

DE, a complex mixture of gases and particles, is currently one of the main components of air pollution. It is now well known that exposure to DE can cause respiratory disorders such as lung cancer,[40] allergic rhinitis,[41] asthma,[41] and chronic obstructive pulmonary disease.[42] However, there are also reports that DEPs enter the circulatory system and translocate to extrapulmonary tissues.[43] These results suggest that exposure to DE can lead to detrimental effects on organ systems other than the lungs. In particular, since the particles enter the circulatory system, maternal exposure to airborne DE can lead to the particles causing damage to the developing fetus as well. In fact, several recent studies in murine models have shown that prenatal DE exposure leads to adverse effects on the reproductive and central nervous systems (Fig. 4).

Effects of Maternal Exposure to DE on Development of the Reproductive System

It has been reported that fetal exposure to DE leads to changes in serum testosterone levels at 3,[44] 4,[45] and 12[44] weeks after birth in mice. In addition, serum testosterone levels have been shown to be correlated with expression of steroidogenic enzyme mRNA, weight of the testes and male reproductive accessory glands, and daily sperm production (DSP).[45] These changes are confirmed in similar studies that showed that maternal DE exposure led to decreased adult expression of steroidogenic factor-1 (Ad4BP/SF-1) and mullerian inhibiting substance (MIS) mRNA[46] as well as decreased DSP at 5 and 12 weeks of age.[44] However, these results appear to be strain dependent as a study comparing the effects of maternal DE exposure among ICR, ddY, and C57BL/6J reported different responses in MIS and Ad4BP/SF-1 among the different strains.[47] Additional measurements of mRNA levels in ICR mice have shown that levels of FSH receptor[44] and steroidogenesis acute

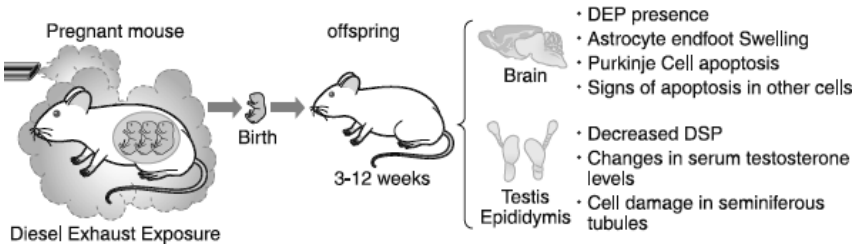

Fig. 4. Maternal Exposure to DE Affects the Central Nervous System and Male Reproductive System

Mice were maternally exposed to diesel exhaust during pregnancy. After birth, offspring were raised in clean air environments. Sampling of the testis, brain, and epididymis took place at various times between 3 and 12 weeks after birth. The results show that the maternal exposure damaged cells and disrupted normal function of the brain[54–56] and male genitals.[44–47] Abbreviations: DEP, diesel exhaust particle; DSP, daily sperm production.

regulatory protein[44] mRNA were increased at 5 and 12 weeks postnatal age, respectively, while 3β-hydroxysteroid dehydrogenase and aromatase, steroidogenic cytochrome P450 (CYP) genes regulated by Ad4BP/SF-1, had decreased mRNA levels in the fetus at 14 days postcoitum.[46]

Maternal exposure to filtered DE, which had 99.97% of the DEPs > 0.3 μm in diameter removed, led to decreased DSP at 12 weeks, increased serum testosterone at 5 weeks, and increased mRNA levels of follicle stimulating hormone receptors, luteinizing hormone, 17α-hydroxylase/C17-20-lyase and 17β-HSD mRNA were reported at 5, 12, and 12 weeks, respectively.[48] Additionally, histological examinations of the seminiferous tubules revealed multinucleated giant cells and partial vacuolation.[48] Watanabe[49] reported that maternal DE exposure and even maternal filtered-DE exposure led to decreased numbers of daily produced sperm, spermatids and Sertoli cells at 96 days age in rats. These data suggest that the most harmful part of DE are gases and particles less than < 0.3 μm in diameter.

The response of female reproductive development to maternal DE exposure is different from the male response. Ad4BP/SF1 and MIS mRNA levels are not changed following maternal DE exposure, but levels of bone morphogenetic protein-15, reported to be related to oocyte development,[50] were significantly decreased.[51] This data suggests that maternal exposure to DE may cause different adverse effects on reproductive development of female fetus offspring. In addition, maternal and postnatal DE exposure in female rats has been shown to enhance proliferation of the rat endometriosis model accompanied by an increase in serum monocyte chemoattractant protein-1 levels,[52] which is consistent with reports regarding cytokine expression in endometriosis in humans and the rat model.[53]

Effects of Maternal Exposure to DE on Development of the Central Nervous System

Since the blood-brain barrier is not fully developed in the fetus, it is believed that DE nanoparticles can pass from maternal circulation into the fetal circulation and enter the fetal brain. This translocation of nanoparticles to the brain has been confirmed in rats.[54] In addition, Sugamata *et al*.[55] observed ultrafine particles in the granular perithelial cells, scavenger cells surrounding cerebral vessels, of mice following prenatal DE exposure. These, and other cells, showed signs of apoptosis, including crescent-shaped vacuoles and caspase-3.

Apoptosis of endothelial cells and stenosis of capillaries were also observed. A subsequent study[56] found a higher number of apoptotic Purkinje cells in mice following DE exposure, which is similar to a symptom associated with autism. These studies highlight the risk of central nervous system disruption in fetal DE exposure.

EFFECT OF PERINATAL ENVIRONMENT TOBACCO SMOKE EXPOSURE

Data from epidemiological studies show that risk for wheezing, attacks of dyspnea, and bronchitis are greater for individuals with fetal and postnatal exposure to ETS than those only postnatally exposed.[57] This suggests that the fetal period is critical for the development of the respiratory system, which concurs with current knowledge about human physiological development.[58] Joad *et al*.[59] exposed rats prenatally and postnatally to either filtered air or sidestream smoke and found that the exposure increased lung sensitivity to methacholine challenge and caused neuroendocrine cell

proliferation. This led the researchers to conclude that perinatal ETS exposure programmed hyperresponsiveness in the respiratory system through pulmonary neuroendocrine cell proliferation. In addition, Wang *et al*.[60] have shown that perinatal and postnatal ETS exposure in monkeys causes a decrease in the T helper type (Th) 1 cytokine interferon-γ and an increase in the Th2 cytokine interleukin-10 with age, which is the exact opposite of the trend in the control group. The researchers hypothesize that the ETS exposure upsets the maturation of Th1/Th2 cytokine balance in favor of the allergy-associated Th2 cytokines.

CONCLUSION

We have reviewed the effects of maternal malnutrition and maternal exposure to DE and ETS. All of these fetal environmental factors have been shown to cause long-term adverse effects on offspring. This is especially concerning during the current period of increased global industrialization, with regions transitioning from impoverished rural areas to prosperous and polluted urban and suburban settings. Early epidemiological data and animal studies suggest that these changes can potentially lead to an epidemic of adult disease. Increased knowledge and public awareness is important in counteracting this possibility.

In addition, the studies of maternal exposure to DE have shown that exposure to airborne pollutants can adversely effects on extrapulmonary tissues, widening the range of targets for the toxic effects of environmental pollutants. In fact, maternal exposure may be more dangerous than adult exposure since the findings reviewed suggest the former allows particles to pass through the developing blood-brain barrier and damage the central nervous system. As a diesel fuel usage has increased with increased industrialization, it has become imperative to fully understand the health effects of this pollutant.

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