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(RESEARCH ARTICLE)

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Teratogenic and biochemical studies of the atypical antipsychotic, Aripiprazole on chick embryo

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Abstract

Aripiprazole is a third-generation atypical antipsychotic drug, typically used to treat schizophrenia, bipolar disorder, and treatment-resistant depression. Although its pharmacological profile has been well-characterized, its developmental toxicity remains poorly understood. Though it is classified as 'Pregnancy Category C' based on animal studies, this drug is the second-most-used antipsychotic drug among pregnant individuals.

The objective of our study was to evaluate the effects of Aripiprazole on chick embryonic development, with a focus on teratogenesis, vascular anomalies, and protein expression. Dose-response tests of a commercial Aripiprazole tablet at concentrations of 5 ppm and 10 ppm were performed on 3 distinct stages of development: HH stages 20-21 (72 hours or 3 days), HH stages 25-26 (120 hours or 5 days), and HH stages 33-34 (192 hours or 8 days). Each inoculation used 100 µl of the appropriate working solution, and the incubation period was 24 hours. The isolated embryos were checked for visible teratogenesis, fluctuations in protein and ALP levels.

The results showed varying degrees of, but irrefutable, teratogenic effects and impact on protein expression at all stages of development. Morphological analysis revealed dose-dependent malformations and growth retardation. The effects of the drug at these doses were observed to decrease as the developmental stage progressed. Tissue and vascular damage were documented, and biochemical evaluations revealed a trend of increase in total protein content with increasing drug concentration. Our findings allow us to conclude that Aripiprazole may not be safe for embryos. Further studies on mammalian models and/or cell lines are required.

Keywords: Aripiprazole; Chick Embryos; Hamburger Hamilton (HH) Staging System; Teratogenesis; Protein Expression

1. Introduction

Currently, we stand at a peak of advocacy and resource availability for mental health issues, with both a need and a rise of new antipsychotic drugs entering the market, each claiming to have fewer side effects than the previous ones. In clinical settings, prescribing any antipsychotic to pregnant patients is of great concern, as antipsychotic drugs have been confirmed to cross the placenta, and data suggests that they have potential neurotoxic effects [1]. A study in 2016 also noted a 100% increment in the frequency of use of antipsychotic drugs during pregnancy. There remains a significant gap in the research regarding the comprehensive effects of antipsychotic medications in pregnant patients, particularly concerning their impact on the developing embryo and fetus [2].

Aripiprazole is a U.S. Food and Drug Administration-approved, third-generation, atypical antipsychotic used to treat treatment-resistant major depression, bipolar disorder, and most commonly, schizophrenia [3]. Off-label, the drug is also

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seen being prescribed to autistic individuals [4, 5]. Among the two most frequently prescribed medications during pregnancy, this agent stands out due to its unique pharmacological profile. Unlike other antipsychotic treatments, it does not induce hyperprolactinemia, thereby preserving reproductive function and minimizing the potential adverse effects on fertility [6]. Aripiprazole is also distinguished from earlier antipsychotic medications due to its mode of action, which involves agonism, partial agonism, and antagonism to both dopamine and serotonin receptors, indicating broader pharmacological uses and fewer evidence-supported side effects, such as excessive weight gain [7]. On a molecular level, Aripiprazole is structurally nonpolar, which prevents it from effectively integrating into the lattice structure of water, resulting in poor aqueous solubility (AS). It is classified as a Biopharmaceutical Classification System (BCS) Class II drug, indicating low aqueous solubility but high cellular membrane permeability [8, 9].

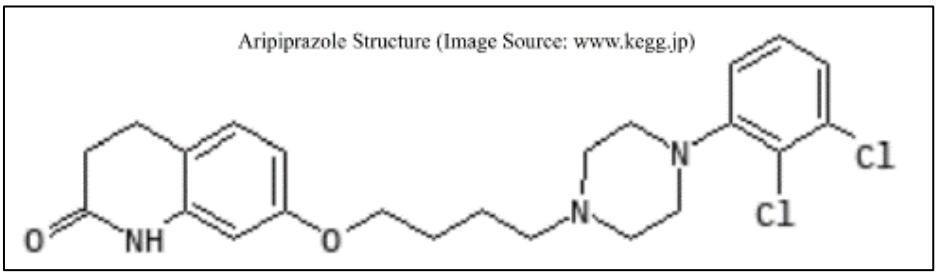


Figure 1 Molecular Structure of Aripiprazole [https://www.kegg.jp/entry/C12564, Last accessed on 23/08/2025]

Aripiprazole is classified as a Pregnancy Category C medication, indicating that animal studies have revealed potential harm to the fetus [10]. This classification underscores the necessity for further research to thoroughly investigate the potential effects of such drugs on fetal development. Addressing this knowledge gap is critical for ensuring maternal and fetal safety during treatment. Studying embryos of *Gallus gallus domesticus* is commonly used in scientific research and biopharmaceutical testing due to the embryo's resemblance to human embryos [11] while adhering to the ethical guidelines governing embryological research. Despite the limitations that chick embryo models impose on research, such as the absence of a placenta for nutrient exchange and the lack of hormonal influence due to maternal metabolism, developmental toxicity testing on chick embryos provides a better understanding of the impact of certain compounds on the development of organisms [12].

The objective of our study was to evaluate the effects of Aripiprazole on embryonic development, with a focus on teratogenesis, vascular anomalies, and protein expression. Teratogenesis refers to structural, functional, or growth-related congenital abnormalities caused by exposure to teratogenic agents [13]. Antipsychotic use, including Aripiprazole, has been associated with neural tube defects, although the precise mechanisms remain unclear and are thought to involve genetic, pharmacological, and environmental factors [14, 15]. Protein expression, reflective of transcriptional and translational changes, was measured using Bradford's colorimetric assay [https://bioanalysis.in/protein-estimation-by-bradford-assay, Last accessed on 23/08/2025] and alkaline phosphatase (ALP) activity. ALP plays key roles in phosphate metabolism and bone development, and its expression is essential for normal embryonic skeleton, abdominal organs, and brain development in avian models [16, 17]. Through these approaches, our study aims to clarify the developmental toxicity profile of Aripiprazole.

2. Materials and methods

Fertilized eggs of *Gallus gallus domesticus* were procured from Venkateshwara Hatcheries Pvt. Ltd., Pune. The procured eggs were cleaned with 70% ethanol, wiped, labeled, and kept in an incubator (REMI® BOD incubator) at 37°C with 70-80% relative humidity (Rh). Embryos of 3 stages were used for studies [18] - HH 20-21 (72 hours or 3 days), HH 25-26 (120 hours or 5 days), and HH 33-34 (192 hours or 8 days).

Aripiprazole (Arpi MT 5 by Torrent Pharmaceuticals Ltd®, Sikkim) was solubilized in DMSO (Dimethyl Sulfoxide) and 1x PBS (Phosphate Buffered Saline) to prepare a stock solution and working solutions of concentrations 5 ppm and 10 ppm. Each egg was treated with 100 µl of the working solution and incubated for 24 hrs. The control eggs and vehicle control eggs with DMSO concentration equivalent to the 10-ppm working solution were incubated for 24 hrs.

2.1. Teratogenesis

Inoculation was done via the air-sac route by the window method for HH 20-21 (3 days) and HH 25-26 (5 days) [18]. Eggs were sealed with Parafilm M and incubated for 24 hours at 37 ℃ at 70-80% relative humidity, alongside control eggs (not treated). After 24 hours, embryos were isolated in 1x sterile Phosphate Buffered Saline (PBS) and checked for teratogenesis.

HH 33-34 (8 days) [18] eggs were treated with 5 ppm and 10 ppm working solutions via chorioallantois membrane (CAM) inoculation by the window method. The treated eggs were sealed with Parafilm M and incubated, alongside control eggs, for 24 hours.

2.2. Protein Biochemistry

Treated and control embryos were isolated and homogenized in sterile chilled 1x PEB (Protein Extraction Buffer) using a Potter-Leveche PTFE tissue homogenizer. Protein estimation was done using Bradford's method [https://bioanalysis.in/protein-estimation-by-bradford-assay, Last accessed on 23/08/2025]. Optical density (OD) was measured at 600 nm using the Siltronic's µC colorimeter 115 for treated and control protein extracts.

2.3. Enzyme Assay

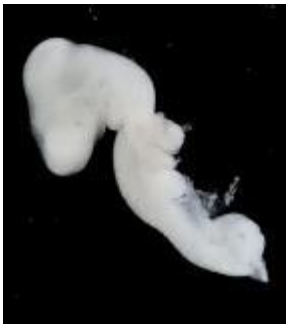
Alkaline Phosphatase (ALP) assay was carried out using an automated sample analyzer called the Moralizer™ AutoQuant 200i. The samples were transferred into labeled cuvettes, which were loaded into the analyzer, and the results were presented on a linked computer. The results were obtained for each sample, and histograms were made to get a quick, visual aid of the results.

3. Results

3.1. Teratogenesis

3.1.1. HH 20-21 (3 days)

Teratogenesis was observed in the embryos treated with 5 ppm and 10 ppm Aripiprazole. The following specific deformities were visible in the embryos of stage HH 20-21(72 hrs.): Embryonic growth reduction(atrophy), neural tube defects, microphthalmia, anophthalmia, degenerated limb buds, and abnormal flexion and torsion.

Control	5 ppm	10 ppm
		
Figure 2 Untreated	**Figure 3** Reduction in Embryonic size, Microphthalmia, Abnormal flexion, Neural tube defects, reduction in somites number	**Figure 4** Microphthalmia, Neural tube defects, Abnormal flexion

3.1.2. HH 25-26 (5 days)

Teratogenesis was observed in the embryos treated with 5 ppm and 10 ppm Aripiprazole. The following abnormalities were visible in the embryos of stage HH 25-26 (120 hrs.): Embryonic growth reduction(atrophy), degeneration of head structures, microphthalmia, anophthalmia, degenerated limb buds, hernia of the ventral body wall - abdominal hernia, and abnormal flexion and torsion.

Control	5 ppm	10 ppm	Vehicle Control

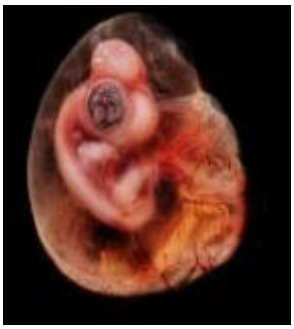
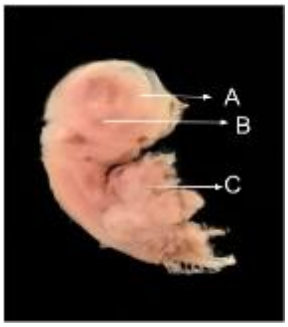
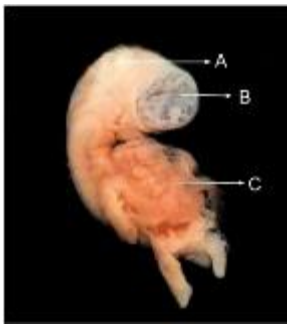
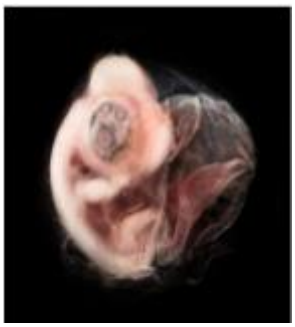

			

Figure 5 Untreated	**Figure 6** A: - degeneration of head structures and brain; B: - distinct optic degeneration; C: - ventral body wall hernia, degenerated limb buds	**Figure 7** A: - degeneration of head structures and brain; B: - distinct optic degeneration; C: - ventral body wall hernia, poorly developed limb buds	**Figure 8** Treated with DMSO

3.1.3. HH 33-34 (8 days)

Hemorrhage was observed in the embryos of stage HH 33-34(172 hrs.), which were treated with 5 ppm and 10 ppm Aripiprazole, when compared with isolated control embryos.

Control	5 ppm	10 ppm

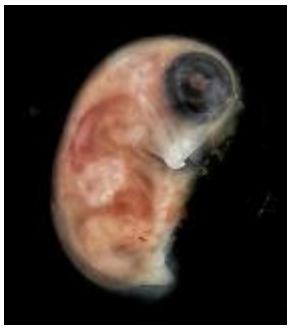
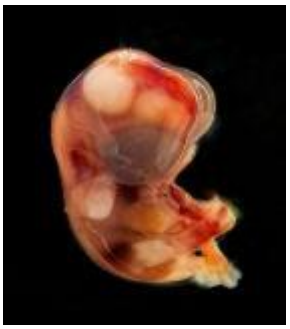

	

Figure 9 No Hemorrhage	**Figure 10** Hemorrhage	**Figure 11** Hemorrhage

3.2. Protein Biochemistry

3.2.1. HH 20-21 (3 days)

Protein quantification studies on Aripiprazole treated embryos showed a decrease in total protein content (5 ppm: 89.7 µg/ml and 10 ppm: 108.7 µg/ml) as compared to control embryos (168.6 µg/ml).

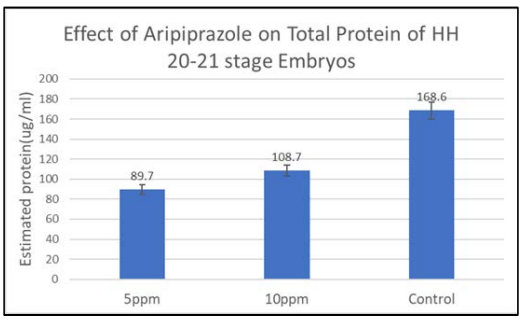


Figure 12 Effect of Aripiprazole on Total Protein of HH 20-21 stage Embryos

3.2.2. HH 25-26 (5 days)

Protein quantification studies on Aripiprazole treated embryos showed a decrease in total protein content (5 ppm: 161.3 µg/ml and 10 ppm: 169 µg/ml) as compared to control embryos (192.1 µg/ml).

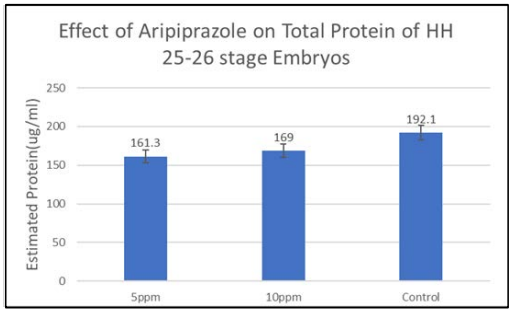


Figure 13 Effect of Aripiprazole on Total Protein of HH 25-26 stage Embryos

3.2.3. HH 33-34 (8 days)

- Total Brain protein

Protein quantification studies on Aripiprazole treated embryos showed an increase in total protein content (5 ppm:195.7 µg/ml and 10 ppm: 252.4 µg/ml) as compared to control embryos (179.3 µg/ml).

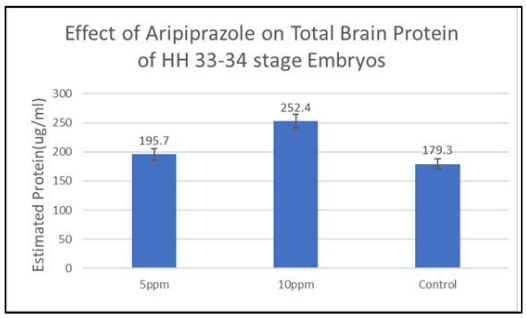


Figure 14 Effect of Aripiprazole on Total Brain Protein of HH 33-34 stage Embryos

- Total Eye protein

Protein quantification studies on Aripiprazole treated embryos showed an increase in total protein content (5 ppm:164.8 µg/ml and 10 ppm: 206.6 µg/ml) as compared to control embryos (113.5 µg/ml).

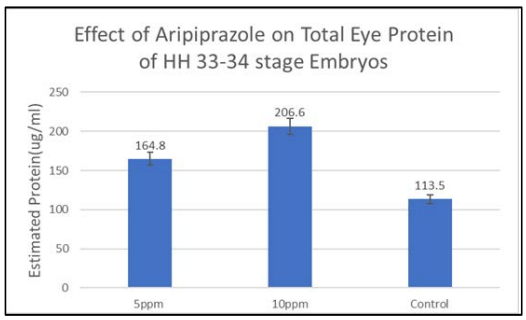


Figure 15 Effect of Aripiprazole on Total Eye Protein of HH 33-34 stage Embryos

- Total Heart protein

Protein quantification studies on Aripiprazole treated embryos showed a decrease in total protein content (5 ppm: 39 µg/ml and 10 ppm: 73.5 µg/ml) as compared to control embryos (85.7 µg/ml).

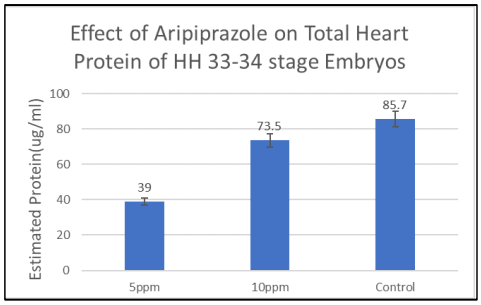


Figure 16 Effect of Aripiprazole on Total Heart Protein of HH 33-34 stage Embryos

3.3. Enzyme Assay (Alkaline Phosphatase-ALP)

3.3.1. HH 20-21 (3 days)

Enzyme assay studies on Aripiprazole treated embryos showed a decrease in alkaline phosphatase levels (5 ppm: 10.9 IU/L and 10 ppm: 30.1 IU/L) as compared to control embryos (67.9 IU/L).

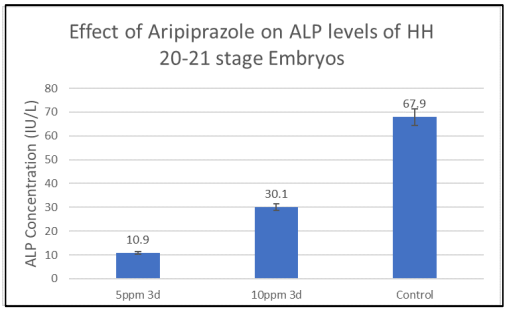


Figure 17 Effect of Aripiprazole on ALP levels of HH 20-21 stage Embryos

3.3.2. HH 25-26 (5 days)

Enzyme assay studies on Aripiprazole treated embryos showed a decrease in alkaline phosphatase levels (5 ppm: 6.3 IU/ L and 10 ppm: 13.9 IU/L) as compared to control embryos (59.6 IU/L).

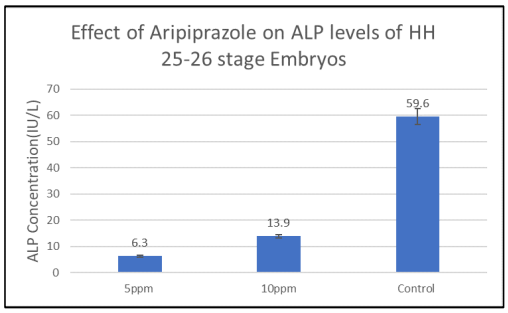


Figure 18 Effect of Aripiprazole on ALP levels of HH 25-26 stage Embryos

3.3.3. HH 33-34 (8 days)

- Brain ALP

Enzyme assay studies on Aripiprazole treated embryos showed an increase in alkaline phosphatase levels (5 ppm: 50 IU/L and 10 ppm: 318.1 IU/L) as compared to control embryos (32.8 IU/L).

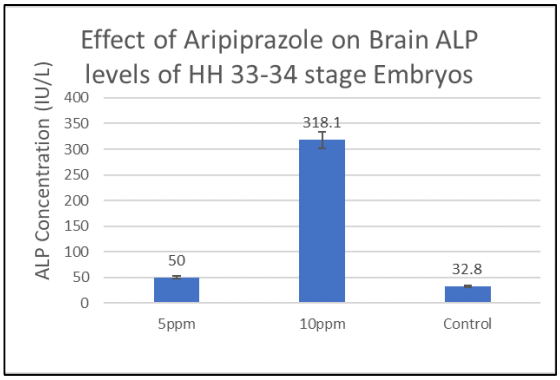


Figure 19 Effect of Aripiprazole on Brain ALP levels of HH 33-34 stage Embryos

- Eye ALP

Enzyme assay studies on Aripiprazole treated embryos showed an increase in alkaline phosphatase levels (5 ppm: 28.1 IU/L and 10 ppm: 159.3 IU/L) as compared to control embryos (23.8 IU/L).

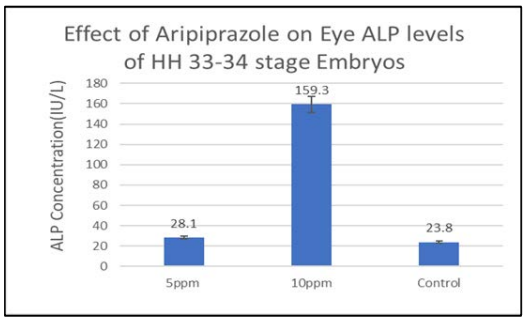


Figure 20 Effect of Aripiprazole on Eye ALP levels of HH 33-34 stage Embryos

4. Discussion

Aripiprazole is a U.S. Food and Drug Administration-approved, third-generation, atypical antipsychotic [3]. Aripiprazole is structurally nonpolar, which prevents it from effectively integrating into the lattice structure of water, resulting in poor aqueous solubility (AS) [8, 9].

To tackle issues with Aripiprazole solubility, we referred to a study involving direct injection of DMSO into the yolk. The results of the study indicated that 0.05 mL (50 µL) per egg did not adversely affect embryonic survival or hatchability [19]. Vehicle control testing showed no morphological differences compared to untreated embryos, confirming that DMSO alone did not contribute to the observed effects.

At concentrations of 5 ppm and 10 ppm, Aripiprazole treated embryos showed notable embryonic changes, including yolk discoloration, degeneration of head structures, neural tube defects, reduction in somites number and embryo degeneration, implicating the teratogenic potential of the drug. Hemorrhage was also observed in treated embryos.

These findings are supported by previous studies, which report that Aripiprazole induces neural tube defects and delays neurogenesis in early-stage chick embryos [8]. Additionally, studies on cortex organoids—models of fetal brain development—have demonstrated adverse effects of Aripiprazole on neural development [10].

In early-stage embryos, at HH 20–21 (3 days) and HH 25–26 (5 days) [18], treated embryos exhibited decreased total protein levels (Figure 12 and Figure 13) compared to controls, indicating interference with protein synthesis during early development. In contrast, HH 33–34 (8 days) [18], treated embryos displayed increased protein levels in brain (Figure 14) and eye (Figure 15) tissues but reduced protein levels in the heart tissue (Figure 16) compared to controls. This indicates that Aripiprazole may differentially affect protein synthesis across tissues to varying degrees. Further more intensive studies are required for a better inference regarding the nuanced effects of Aripiprazole.

For HH 25–26 (5 days) embryos, protein estimation was specifically conducted on the head region, considering previous evidence that Aripiprazole induces abnormal morphogenesis in cortex organoid models [20]. At HH 33–34 (8 days), protein estimation was extended to brain, eye, and heart tissues to assess tissue-specific effects.

Atypical antipsychotic drug treatment usually elevates ALP levels [21]. Our ALP activity assays revealed stage-dependent effects. In HH 20–21 (3 days) and HH 25–26 (5 days) [18] embryos, treated samples exhibited markedly reduced ALP levels (Figure 17 and Figure 18). Interestingly, at HH 33–34 (8 days) [18], brain and eye tissues from embryos exposed to Aripiprazole exhibited significantly elevated ALP levels (Figure 19 and Figure 20), surpassing those in controls. Elevated ALP levels have been associated with altered bone metabolism and hepatotoxicity in previous studies associated with chick embryo [22].

5. Conclusion

Our studies indicate that Aripiprazole induces teratogenesis, alters total protein content and ALP levels in HH stages 20-21 (3 days) and HH stages 25-26 (5 days) of the chick embryo via reduction in somites number, poorly developed brain, and optic degeneration. The extent of teratogenesis is reduced in HH stages 33-34 (8 days) of the chick embryo, as the only visible change seen is hemorrhage.

Our findings suggest Aripiprazole may exert teratogenic effects. This raises concerns about the drug's safety during early developmental windows and supports the need for further mechanistic investigation.

Further studies need to be done, preferably on mammalian models and cell lines to better understand the action of Aripiprazole on neurotransmitters and its effect in later stages of development.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest to be disclosed.

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