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**ORIGINAL ARTICLE**

**EFFECT OF ARSENIC ON BIOENZYMOLOGICAL STUDIES IN FRESH WATER FISH,  
LABEO ROHITA**

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**ABSTRACT**

In the present study, the acid phosphatase (ACP), alkalainphosphatase (ALP), aspartate amino transferase (AST) and alanine aminotransferase (ALT) levels were observed in both control and arsenic exposed fish, *Labeo rohita*. The present study showed that the the acid phosphatase (ACP), alkalainphosphatase (ALP), aspartate amino transferase (AST) and alanine aminotransferase (ALT) were increased in liver and kidney tissues of arsenic exposed fish, *Labeo rohita*. The present study concluded that the arsenic compounds could damage the liver and kidney of the fresh water fish, *Labeo rohita*.

**Key words:** Arsenic, ACP, ALP, AST, ALT, Liver, Kidney

**1. INTRODUCTION**

Environmental pollution is caused by the development of industries, technology and informal settlements does, however, threaten many freshwater ecosystems. Environmental pollution not only cause a decrease in water quality, but it subsequently affects all living organisms in that system. Therefore, it is necessary to not only identify and manage these pollution sources, but also to maintain their effects on the health of aquatic environment. Human activities are mainly responsible for promoting the pollution in the environment by the way of introducing unwanted toxic compounds (Bryan, 1976).

Heavy metals are widely found in natural environment mostly representing severe health hazards in organism [Bamennan and Schiesty, 1996]. The toxicological effects of pollution are due to their high persistence and accumulation in the organisms [Goyer, 1996]. Although suitable concentration of heavy metals play a vital role in metabolic pathways when their concentration exceed the threshold level, they act as physiological biochemical and behavioral inhibition in the organisms.

Metals are elements found naturally in aquatic ecosystems due to various processes such as weathering and erosion (Viljoen,

1999). Some of these metals are essential to living organisms in trace amounts (for example copper and zinc). Essential trace elements have a narrow optimal concentration range for growth and reproduction, and both excess and shortage can be detrimental to organisms (Pelgromet *al.*, 1994), with unusually high concentrations becoming toxic to aquatic organisms (Wepeneret *al.*, 2001). Other metals (for example cadmium and lead) have unknown biological function (Seymore, 1994). Cadmium is major contaminants of aquatic environments (Mungeret *al.*, 1999) that are toxic towards aquatic organisms (Witeskaet *al.*, 1995) even at concentrations found in natural waters (Pelgromet *al.*, 1994). Metals are present in very low concentrations in natural aquatic ecosystems (Nussey, 1998). The most important heavy metals in water pollution are zinc, copper, lead, cadmium, mercury, nickel and chromium (Seymore, 1994; Viljoen, 1999). Metal uptake by aquatic organisms is a two-phased process, firstly involving rapid adsorption or surface binding, followed by a slower transport into the cell interior. Transport of metals into the intracellular section may be aided by either diffusion of the metal ion across the cell membrane or by active transport by a carrier protein (Brezoniket *al.*, 1991; Wepeneret *al.*, 2001).

The health of fish may be affected, either directly through uptake from the water, or indirectly through their diet of vegetation, invertebrates or smaller fish. Metals released into

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aquatic ecosystems, are responsible for several fish physiology irregularities (Sehgal and Saxena, 1986). They can also disturb the ionoregulatory mechanism in aquatic organisms (Hansen *et al.*, 1996). All of these effects of heavy metals usually affect fish negatively leading to stress and eventually, in most cases, death.

The heavy metals are constantly polluting natural waters and the adverse effects are manifold on living organisms including economically important fishes. They are also responsible for various disturbances in physiological and biochemical parameters of fish (Shaffi, 1979). Arsenic is widely distributed in soils, sediments, water, air and living organisms. Arsenic concentrations found in natural waters range from less than 0.5 mg/l to more than 5000 mg/l. Extreme concentrations are rare but are most frequently found in groundwater (Smedley *et al.*, 2002).

Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish (Tulasiet *al.*, 1992). The heavy metal in the tissue of fishes may cause various physiological defects and mortality (Torres *et al.*, 1987). The fishes which are largely being used for the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Dautrempuis *et al.*, 2004).

Liver is one of the most multifaceted and active organs in higher animals. In a vertebrate body, the liver is the most important target organ as it is the chief metabolic and detoxification center (Abbasi, and Sujata Krishnan, 1993). It is the site for numerous and varied metabolic activities, including synthesis of bile which contains bile salts, bile pigments, cholesterol and lecithin. The kidney as an organ is mainly concerned with the removal of waste materials. The pathological effects of heavy metals and pesticides on kidney of various animals have been studied by several workers (Rajamanickam 1992). In fish, as in higher vertebrates the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen containing waste products from the metabolism such as ammonia and urea.

## 2. MATERIALS AND METHODS

*Labeo rohita* was collected from the fish farm located in Pinnalur, 20 km away from the overnment Arts College, C.Mutlur. The collected fishes without least disturbance were transported in polythene bags filled half with water without any disturbances. About 100 fishes were put in each bag and water was well aerated, using pressurized air from a cylinder. These modes of transit have proved successful, since there was no mortality in all consignments throughout the course of this study. To evaluate the acute toxicity static renewal toxicity test were conducted according to the methods recommended by American Public Health Association (APHA) (1960). In the present investigation the toxicity of Arsenic trioxide the median lethal concentration (LC50) of Arsenic f analyzed. The LC50 is statistically estimated to the concentration of toxic material in water that kills 50 per cent of the test species, under experimental conditions during a specific time interval (Sparague, 1971). The LC50 was used, because, the

concentration required affecting the response in 50 percent of the test animals is more reproducible than any other value (Pickering and Handerson, 1966). Preliminary observation showed that beyond 30 ppm of Arsenic trioxide all the test fishes died. Therefore the concentration of arsenic trioxide falling off within 1 to 30 ppm was prepared. Ten number of test fishes were introduced to conform narrow range of concentration viz., 1, 2.5, 5.0, 7.5, 10.0 12.5, 15.0, 17.5, 20.5, 23.0, 25.0, 27.5, 30.0 ppm of arsenic solutions. The behavioral responses of the fish at various concentration of Arsenic trioxide were observed at regular intervals to ascertain the impact of the arsenic toxicity on the organism. Individuals in the test medium, which showed no responses to stimulation and those without opercular movement, were removed quickly to avoid cannibalism among the fish. In all tests, mortalities were recorded at 24, 48, 72 and 96 hours. determined by following the method of Finney (1971). Sublethal studies are helpful to assess the response of the test organisms under augmented stress caused by metals. The LC50 values were one tenth of the 96 hr LC50 sublethal concentration respectively. 96 hr LC50 value for arsenic was found at 1.89 ppm. Hence the one tenth of 96hr LC50 value (1.89 ppm) was selected for the present investigation as sublethal concentration for the period of 7 days. The experimental fish were exposed to sublethal concentration of arsenic for a period of 7 days. The control and experimental fish were dissected out at the end of each period of exposure and the selected organs such as liver and kidney were dissected out for biochemical studies.

### Biochemical estimations

#### Estimation of acid and alkaline phosphatase in tissue

Activities of acid and alkaline phosphatase were estimated by the following procedure adopted by Tennis Wood *et al.*, (1976).

#### Estimation of acid phosphatase

The isolated whole tissues were homogenized in glass homogenizer using 10ml distilled water. This content was centrifuged at 3000 rpm for 10 minutes. In a clean test tube 0.5 ml of supernatant was taken and 0.5ml of the substrate solution (p-nitrophenyl phosphate) and 0.5 ml of 0.1 N citrate buffer were added. The test tube with the above solution was kept in a water bath maintained at  $37 \pm 38^\circ\text{C}$  for 30 minutes. After completion of 30 minutes, the reaction was arrested in the extracts by adding 3.8 ml of 0.1N sodium hydroxide. The colour formed at the end was read at 415 nm in UV spectrophotometer. Phenol was used to construct the standard graph. The values are expressed in  $\mu$  moles of phenol liberated / min / 100 mg of protein.

#### Estimation of alkaline phosphatase

The isolated whole tissues were homogenized using 10 ml of distilled water. This content was centrifuged at 3000 rpm for 10 minutes. In a clean test tube 0.5 ml of supernatant was taken and 0.5 ml of the substrate solution (p-nitrophenyl phosphate) and 0.5 ml of glycine buffer were added. The test tube with above solutions was kept in a water bath maintained at  $37 \pm 38^\circ\text{C}$  for 30 minutes. After completion of 30 minutes, the reaction was arrested in the extract by adding 10 ml of 0.02N sodium hydroxide. The colour formed at the end was read at 415 nm in UV spectrophotometer. Phenol was used to construct

the standard graph. The values are expressed in  $\mu$  moles of phenol liberated / min /100 mg of protein.

#### Estimation of aspartate amino transaminase (AST) and alanine amino transaminase (ALT)

The activity of aspartate aminotransaminase (AST) and alanine amino transaminase (ALT) were determined by adopting the method of King (1965). 1ml of substrate (AST -1.33g of L-aspartic acid and 15mg of  $\alpha$ -ketoglutaric acid was dissolved in 20.5 ml of phosphate buffer and 1N sodium hydroxide to adjust pH 7.5 and make up to 50 ml with buffer; 1.78g of DL - alanine and 30mg of  $\alpha$ -ketoglutaric acid were dissolved in 20ml of phosphate buffer. The pH was adjusted to 7.5 with 1N sodium hydroxide and made upto 100ml with buffer. A few drops of chloroform (added) was taken in a clean test tube and it was incubated for 5 minutes at 37°C. Then 0.2ml of tissue homogenate was added in the test tube and incubation was maintained for an hour in the case of AST and 30 minutes for ALT. The reaction was arrested by adding 1.0 ml of DNPH reagent and then the tubes were kept at room temperature for 20 minutes. Then 10 ml of 0.4 N Sodium hydroxide solution was added and the colour developed was read at 520 nm against a reagent blank in UV Spectrophotometer (Bausch and Lomb). Pyruvic acid was also treated in a similar manner for the standard. The activities of liver and kidney AST and ALT were expressed as IU/L.

#### Statistical analysis

Statistical significance was evaluated by using ANOVA followed by Duncan Multiple Range Test (DMRT) Duncan (1957).

### 3.RESULTS

The level of acid phosphatase exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of arsenic. The acid phosphatase in liver tissue was found to be increased for 7 days. The acid phosphatase level in kidney exhibited a remarkable change from the mean control level, when the fish were exposed to sub-lethal concentration of arsenic. The acid phosphatase in the kidney tissue was found to be increased for 7 days (Fig.1).

The level of alkaline phosphatase exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of arsenic. The alkaline phosphatase in liver tissue was found to be increased for 7 days. The alkaline phosphatase level in kidney exhibited a remarkable change from the mean control level, when the fish were exposed to sub-lethal concentration of arsenic. The alkaline phosphatase in the kidney tissue were found to be decreased for 7 days (Fig.2).

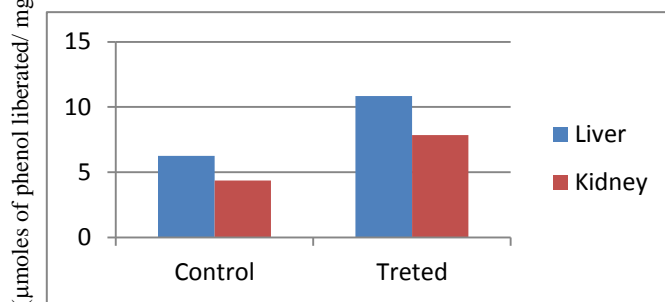
The level of aspartate aminotransferase exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of arsenic. The aspartate aminotransferase in liver tissue was found to be increased for 7 days. The aspartate aminotransferase level in kidney exhibited a remarkable change from the mean control level, when the fish were exposed to sub-lethal concentration of arsenic. The aspartate aminotransferase in the kidney tissue were found to be increased for 7 days (Fig.3).

The level of alanine aminotransferase exhibited remarkable changes in their level from a mean control level, when the fishes were exposed to sub-lethal concentration of arsenic. The alanine aminotransferase in liver tissue was found to be increased for 7 days. The alanine aminotransferase level in kidney exhibited a remarkable change from the mean control level, when the fish were exposed to sub-lethal concentration of arsenic. The alanine aminotransferase in the kidney tissue were found to be increased for 7 days (Fig.4).

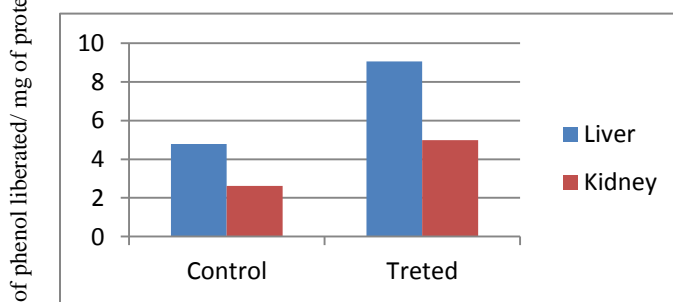
### 4.DISCUSSION

Heavy metals are known for their strong action on biological tissue. Metal ions once absorbed in to the body are capable of reacting with variety of active binding sites and then disturbing the normal physiology of an organism which may lead to the death of the organisms. The toxic effect of heavy metal on the enzyme system depends on the toxicants to react with ligands. The heavy metal may cause injury to the organism and the damaged tissues shall dysfunction which results in altered enzyme activity. Thus enzyme bioassay can provide diagnostic tool to asses a change or damage caused to organism due to administration heavy metal (Harper *et al.*, 1975).

**Fig.1. The level of acid phosphatase (ACP) in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic**



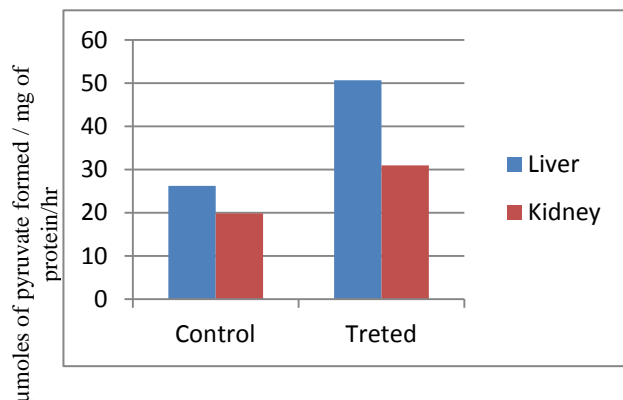
**Fig.2. The level of alkaline phosphatase (ALP) in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic**



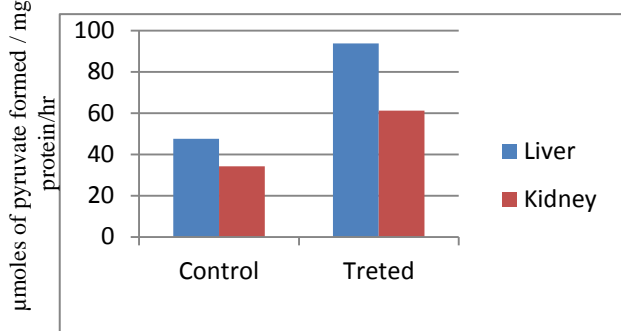
Acid and alkaline phosphatases are known as an inducible enzyme and their activity goes up when the tissues were intoxicated with variety of toxicants (Leland, 1983). Acid and alkaline phosphatases also serve as diagnostic tool to assess toxicity of chemicals in the living organisms (Harper 1991). In the present study the level of acid and alkaline phosphatase activity increased in the liver and kidney tissue of *Labeo rohita* exposed with arsenic for 7 days. These results suggest that increased level of acid and alkaline phosphatase might be due to toxic effect of arsenic. These increased activities can be attributed to the destruction of cell membrane and lysosomes,

which in turn leads to hepatic damage. The increased level of acid phosphatase activity suggested that the involvement of lysosome in metal toxicity. Alkaline phosphatase is involved in the synthesis of nuclear protein, nucleic acid and phospholipids. These enzymes are associated with transmembrane transport mechanism, ion transport, maintenance of ionic strength cell growth in the organ (Moog, 1946).

**Fig.3. The level of aspartate aminotransferase (AST) in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic**



**Fig. 4. The level of alanine aminotransferase (ALT) in the selected tissue of fresh water fish *Labeo rohita* exposed with sub-lethal concentration of arsenic**



Significant increases of acid and alkaline phosphatase were reported in *Cirrhinus mirigala* exposed to lead acetate (Ramalingam *et al.*, 2000). Changes in these enzyme activities were observed in *Channa punctatus* exposed to mercuric chloride (Jeelani and Shaffi, 1989). James *et al.*, (1992) reported that the increased in acid phosphatase activity has been reported in liver tissue of *Oreochromis mossambicus* exposed to heavy metal. Ramesh *et al.*, (1993) acid phosphatase increased in the gill and liver tissue of *Oreochromis mossambicus* exposed to nickel. Hota and Pradha (1994) reported that a significant increase in the level of acid and alkaline phosphatase activities in *Channa punctatus* exposed to mercuric chloride. Sreenivasan *et al.*, 2011 reported that the impact of cypermethrin on the variations in the acid phosphatase (ACP) and alkaline phosphatase (ALP) in the gills *S. hydrodroma*. Lamaire *et al.* (1991) studied acid and alkaline phosphatase (ALP) in fish exposed to malathion.

Heavy metal constitutes an important class of toxic substance which are encountered in numerous occupational and environmental circumstances. The heavy metal released the aquatic and terrestrial media by a variety of anthropogenic

activities, and industrial uses (Mary Chandravathy and Reddy, 1995). With some of these interactions there is high reactivity involving a high degree of inhibition of the specific enzyme that accounts for the effects on the whole animal.

Aspartate aminotransferase (AST) is responsible for transferring amino group from aspartate to 2-βglutamic acid forming glutamate and oxaloacetate. The rise in AST level is virtually responsible for all types of tissue damage (Tiwari and Sivastava, 2001). Alanine aminotransferase (AST) is responsible for transferring amino group from alanine to 2-ketoglutaric acid forming glutamate and pyruvate. It is well known for tissue damage and its level rises higher in most types of hepato cellular damage (Tiwari and Srivastava, 2001). Alterations in AST and ALT level are the biomarker for assessing the toxicity of heavy metals (Martin *et al.*, 1981). From this point of view, the present study has been designed to observe Aspartate aminotransferase and Alanine aminotransferase in the gill, liver and kidney tissues of fish, *Labeo rohita* exposed with arsenic for 7 days of exposure

In the present study, the level of AST and ALT activity increased in the gill, liver and kidney tissues of *Labeo rohita* exposed to arsenic for 7 days. This result may be due to necrosis, which causes increase in the permeability of cell membrane resulting in the damage of tissues. Similar results made reported by Hwang *et al.*, (2000).

They reported that the level of AST and ALT activities are increase due to heavy metals in chronic liver damage. The activity of AST and ALT can be used to indicate the tissue damage of liver and kidney [Nemsoc and Boross, 1982]. Hori *et al.*, (2006) have observed the level of AST in the liver tissue of *Brycon cephalus* exposed with phenol. Khan *et al.*, (1993) reported disturbances in the liver function after heavy metal exposure reported the increase in the AST and ALT activities in the serum of animal exposed to lead. Hori *et al.*, (2006) have observed the level of AST in the liver tissue of *Brycon cephalus* exposed with phenol.

Several investigators also reported that heavy metal intoxication showed a significant increase in AST and ALT activities in the liver tissue of animals (Rana *et al.*, 1996; Khandelwal *et al.*, 2002). The elevated level of AST and ALT indicate stopped up transmutation where feeding of amino acids into the TCA cycle occurs in order to cope up the energy crisis during cypermethrin toxicity (Philip *et al.*, 1995). The significant increase of these enzymes in the tissues seems to indicate possible dysfunction, taking place in the tissues of animals (Casilla *et al.*, 1983).

Sharma (1999) has reported that similar pattern of increase in AST and ALT in the liver tissue of *Channa batrachus* exposed to pesticides. Mary Chandravathy and Reddy (1991) have reported that the elevation of AST and ALT in the gill and brain tissues of *Anabas scandens* exposed to lead nitrate. Usha and Raj (1993) have reported the increase in the level of AST and ALT in the animals exposed to vanadium.

The transaminases GOT and GPT (entering the blood after the cell necrosis of certain organs) can be used to indicate the tissue damage of the liver and kidney (Nemcsó and Boross, 1982). Alterations in the activity of alanine and aspartate transaminase enzymes will be reflected on the energy yielding TCA cycle

and nitrogen metabolism. They also influence the gluconeogenic process and any change in the transaminase activity can be correlated with the protein and carbohydrate metabolism and thereby help in analysing the metabolic shifts (Beyer *et al.*, 1996).

Ghousia Begum (2005) has been reported that alanine and aspartate aminotransaminase were enhanced in hepatic and gill tissues. The elevated activities of both the transaminases (AAT and ALAT) indicate stepped up transamination where feeding of amino acids into the TCA cycle occurs in order to cope with the energy crisis during cypermethrin stress (Philip *et al.*, 1995).

The increase in activity of transaminase and decrease in protein content were observed in hepatic and branchial tissues of *C. batracus* exposure to cypermethrin (Ghousia Begum, 2005). Various investigators report similar findings such as the teleost fish, *Anabas testudineus* treated with thiouracil resulted in increased activity of AST and ALT enzymes (Padmaja Nair and Oommen, 1998).

Usha and Raj (1993) have reported the increase in the level of AST and ALT enzymes as a specific indication of hepatic cell damage and found them to be time and dose dependent. The transaminases function at the junction between the carbohydrate and protein metabolism by interconverting the strategic compounds *viz.*, ketoglutarate, pyruvate and oxaloacetate on one hand and alanine, aspartate and glutamate on other hand.

Ayyanna and Yellamma (1992) have observed the utilization of the reserve food materials like carbohydrate and protein and an increased transaminase activity in the fish *Cirrhinus mrigala* exposed to sublethal concentration of ambient urea. The aspartate and alanine aminotransferase activity were significantly increased in the fish *Labeo rohita* exposed to sublethal concentration of ambient urea (Rajyasree and Neeraja, 1989).

Rao and Ranganathan (1975) has reported that the significant increase of transaminases level in human with acute hepatic disease was due to the damage of parenchymal cells and particularly the glutamic pyruvic transaminase level is the more specific indicator of liver damage. Mukhopadhyay *et al.* (1982) have stated that an increased transaminase activity in the liver of *Clarius batrachus* was due to the exposure to sublethal concentration of carbofuron is compatible with liver damage. The fish *Anabas testudineus* treated with sublethal concentration of phosphamidon exposure showed significant increase in transaminase activities in liver and kidney tissues (Ganguly *et al.*, 1997).

Karan *et al.*, (1998) reported that increased activities of AST and ALT have been observed in gill tissue of *Cyprinus carpio* exposed to copper sulphate and De Smet and Blust, (2001) reported that increased activities of AST and ALT have been observed in gill tissue of *Cyprinus carpio* exposed to cadmium. Vutukuru *et al.*, (2007) reported that the arsenic alters the AST and ALT level in *Labeo rohita*. The exposures to metals like arsenic and chromium may lead these metals to accumulate in the liver. Yang *et al.*, (2003) reported significant increase in transaminases (AST and ALT) activity in fish exposed to arsenic could be due to possible leakage of enzymes across damaged plasma membranes and/or the increased

synthesis of enzymes by the liver. Yang *et al.*, (2003) studies also demonstrated an increased activity of ALT and AST and hepatocyte ultra structure of common carp, *Cyprinus carpio* after gallium exposure. Mukhopadhyay and Dehadrai (1980a,b) noticed various biochemical changes such as GOT and GPT in the air-breathing catfish, *Clarius batrachus* under sublethal concentration of malathion exposure.

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