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ORIGINAL RESEARCH

Effect of arsenic (As) and lead (Pb) on glycogen content and on the activities of selected enzymes involved in carbohydrate metabolism in freshwater catfish, *Heteropneustes fossilis*

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Abstract Heavy metals show a wide range of effect on fishes, out of which arsenic (As) and lead (Pb) are among the leading heavy metal toxicants. These heavy metals are known to alter different biochemical parameters, including glycogen level, in different tissues of fishes. Glycogen level in fish serves as the main source of energy; hence, in this study, the acute toxicity test of As and Pb and their effect on the glycogen content and the enzymes involved therein (glycogen phosphorylase, glycogen synthase, hexokinase, phosphofructokinase and pyruvate kinase) were studied in the liver and muscle tissues of *Heteropneustes fossilis*. The 96 h LC_{50} values of As_2O_3 and $PbCl_2$ on *H. fossilis* were found to be 35.09 ppm and 66.20 ppm, respectively. On acute exposure to 96 h LC_{50} values of As_2O_3 and $PbCl_2$, the glycogen concentration showed a gradual decrease in both liver and muscle tissues of *H. fossilis*. However, on chronic exposure (LC_{50}/20th ppm), the glycogen content in liver and muscle of *H. fossilis* was depleted till 20 days; whereas after 30 days, the glycogen level was recovered in both the tissues. The activities of glycogen metabolic enzymes (glycogen phosphorylase and glycogen synthase) and few selected glycolytic enzymes (hexokinase, phosphofructokinase and pyruvate kinase) were also altered in *H. fossilis* when exposed to acute and chronic concentration of As_2O_3 and $PbCl_2$. Our present results showed that As and Pb induced toxicity stress on the catfish, *H. fossilis*, which might have caused to alter the carbohydrate metabolism in the fish.

Keywords Arsenic · Lead · *H. fossilis* · Glycogen · GPase · GSase

Introduction

Freshwater gets contaminated with different pollutants and is a matter of concern (Vinodhini and Narayanan 2009). Among the various water pollutants, heavy metals pose a great threat to fishes. The natural aquatic systems are contaminated with heavy metals released from domestic, industrial, and other man-made activities (Velez and Montoro 1998). These heavy metals cause the greatest threat to the health of the aquatic ecosystem (Ohe et al. 2004; Govind and Madhuri 2014), as well as human health (Wennberg 1994). Arsenic (As) and lead (Pb) are recognized as the leading toxicants worldwide (Akhtar et al. 2013), and these heavy metals are accumulated in different tissues, and the rate of their accumulation is in the order of muscle > liver > gill for As, and of gill > liver > muscle for Pb (Thang et al. 2017). These heavy metals cause serious damage to aquatic life, including fishes (Karbassi et al. 2006).

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Glycogen is the main source of energy for animals and its content in the liver and muscle indicates the health of the fish. The rate of glycogen synthesis is a function of the relative activities of glycogen synthase (GSase) and glycogen phosphorylase (GPase). Both GPase and GSase exist in two interconvertible forms, active (*a*) and less active (*b*). GPase *a* catalyzes the rate-limiting step of glycogenolysis, and control glycogenesis (Connett and Sahlin 1996). Both As and Pb decrease the level of glycogen in *Oreochromis mossambicus*, and *Labeo rohita* (James et al. 1996; Aruljothi et al. 2013), and a higher concentration of As induces glycogenolysis in the liver of *Channa punctatus* (Haque et al. 2009). Glycogenolysis is catalysed by As in rabbit muscle by producing unstable glucose-1-arsenate (Klein et al. 1982); whereas, Pb decreases the activity of GSase in rat liver, thus decreases the glycogen content (Hacker et al. 1990).

Glucose is oxidized via the glycolytic pathway for the production of ATPs. Glycolytic pathway is inhibited by As and Pb by inhibiting hexokinase (Lagunas 1980; Yun and Hoyer 2000) and pyruvate kinase activity (Lepper et al. 2010) in a rat model. The sub-lethal exposure of Pb decreases the activity of phosphofructokinase activity in the diencephalon, cerebrum, medulla oblongata, and cerebellum of *L. rohita*, *Clarias batrachus* and *C. punctatus* (Raibole et al. 2013).

The Indian catfish, *Heteropneustes fossilis* (Bloch, 1794), is one of the most economically important food fishes in the Indian subcontinent and Southeast Asian region. This is primarily a fish in ponds, ditches, beels, swamps, and marshes, but sometimes found in muddy rivers (Jha and Rayamajhi 2010; Froese and Pauly 2018). It is hardy and amenable to a high stocking rate and utilizes atmospheric oxygen for respiration (Vijaykumar et al. 1998; Haniffa and Sridhar 2002). *H. fossilis* is an exceptionally sturdy and long-lived species; therefore, it served as the best model of study. Hence, in the present study, the effect of As and Pb (both acute and chronic concentrations) on glycogen content and the activities of selected enzymes involved in glycogen metabolism (GPase and GSase) and glucose oxidation (hexokinase, phosphofructokinase, and pyruvate kinase) in both liver and muscle tissues of *H. fossilis* were investigated.

Materials and methods

Chemicals

As_2O_3 (A1010), $PbCl_2$ (268690), Fructose-6-phosphate (F3627), Aldolase (A2714), Triose phosphate isomerase (T6258), Glycerophosphate dehydrogenase (G6751), Glucose 1,6-bisphosphate (G6893), Phosphoglucomutase (P3397) and UDP-glucose (U4625) were purchased from Sigma-Aldrich, St. Louis, USA. HCl (H0090), Sucrose (S2610) and KCl (P0240) were purchased from Rankem, Haryana, India. KOH (GRM1015), Na_2SO_4 (MB209), NaOH (RM467), Tris (RM262), EDTA (RM3915), $MgCl_2$ (MB237), 2-mercaptoethanol (MB041), Triton X-100 (RM845), D-glucose (MB037), $NADP^+$ (RM392), ATP (RM439), G6PDH (RM7066), NADPH (RM 576), Imidazole (RM1864), Phosphoenolpyruvate (RM9768), NADH (RM393), ADP (RM437), $MgSO_4$ (GRM1281), NaF (GRM7502), EGTA (MB130), PMSF (MB144), KH_2PO_4 (MB050), K_2HPO_4 (RM168), AMP (CMS438), Glycogen (RM9593), Caffein (RM1056), Pyruvate kinase (RM7427), Glucose 6-phosphate (RM378), Comassie Brilliant Blue (RM1219) and H_3PO_4 (AS011) were purchased from Himedia, Mumbai, India. LDH (53110) was purchased from Super Religare Laboratories Limited, Mumbai India.

Fishes

The experimental fish, *H. fossilis*, with an average weight of 25 ± 2 g and length of 15 ± 2 cm, were procured from commercial sources and acclimatized to laboratory conditions in dechlorinated water for 15 days in a plastic tank. The acclimatized fishes were then used for subsequent experiments.

LC_{50} determination

Toxicity tests were performed in accordance to the standard methods mentioned in the Organization for Economic Co-operation and Development (OECD 1992). These tests were carried out in plastic tanks containing 8 L of water and 10 fishes for each group. Following the preliminary screening, different

concentrations of As_2O_3 (25 ppm to 50 ppm) and $PbCl_2$ (50 ppm to 100 ppm) were used for bioassay with an independent simultaneous maintenance of control. Feeding was stopped 24 h prior to the treatment and during the experiment. The fishes were exposed to different concentrations of As_2O_3 and $PbCl_2$ for 96 h, and the mortality percentage was recorded at 24 h, 48 h, 72 h and 96 h post exposure. LC_{50} values for As and Pb were calculated by Probit analysis method using Statistical Package for the Social Sciences (SPSS) 17.0 software.

The fishes were then treated with both acute (35.09 ppm As_2O_3 and 66.20 ppm $PbCl_2$) and chronic (LC_{50}/20th ppm of both As_2O_3 and $PbCl_2$) concentrations of As and Pb for the estimation of glycogen content and to study the activities of the selected enzymes (glycogen phosphorylase, glycogen synthase, hexokinase, phosphofructokinase and pyruvate kinase) of *H. fossilis*.

Estimation of glycogen content

The glycogen content in different tissues of *H. fossilis* was estimated following the method as described by Seifter et al. (1949). In brief, 1 g of fish tissues was taken in 3 ml of 30% KOH solution and boiled for 20 min. After adding 2 ml of saturated Na_2SO_4 and 3 ml of 95% ethanol, the homogenate was boiled for 20 min and centrifuged at 3000 g for 5 min. The pellet was dissolved in 2 ml of water and 6 ml of 90% ethanol and boiled for 10 min. The precipitate was redissolved in 3 ml water and 3 ml of 2 N HCl and was boiled for 30 min. Prior to the estimation of glycogen, the solution was neutralized by adding 3 ml of 1 N NaOH and used for the estimation of glycogen. For the estimation of glycogen, 1 ml of the neutralized solution was taken and mixed with 3 ml of 0.2% anthrone reagent (prepared in concentrated H_2SO_4) and boiled for 15 min. After cooling down, the OD was taken at 620 nm using a spectrophotometer (Carry 60, Agilent), and the glycogen content was estimated against a standard glycogen graph.

Transmission electron microscopy (TEM) study

Glycogen content in the liver and muscle tissues of *H. fossilis* was visualized by TEM study. In brief, after treatment, the liver and muscle tissues were cut into small pieces (1 mm × 1 mm) and were fixed in Karnovsky's fixative for 4 h (David et al. 1973). The samples were then washed thoroughly in 0.1 M cacodylate buffer and post-fixation was carried out in 1% osmium tetraoxide for 1 h at 4 °C. The samples were dehydrated in ascending grades of acetone (30%, 50%, 70%, 80%, 90%, 95%, and dry acetone). The acetone was cleared off from the samples by using propylene oxide for 30 min. Embedding of the tissues was carried out in Araldite embedding medium using beam capsule. The embedded blocks were kept at 50 °C in an embedding oven for 24 h at 60 °C. Ultra-thin sections (600–800 Å) were cut in a RMC Ultra-Microtome and the sections were then stained using 50% alcoholic solution of uranyl acetate for 10 min at room temperature in the dark, followed by lead nitrate for 5 min (Reynolds 1963). The stained sections were examined using JOEL JEM 2100 Transmission Electron Microscope at an accelerating voltage of 80 kV.

Enzyme assay

Tissue processing

For assaying the activities of GPase (EC 2.4.1.1) and GSase (EC 2.4.1.11), a 10% tissue homogenate was prepared in a homogenizing buffer containing 20 mM Imidazole–HCl buffer (pH 7.2), 100 mM NaF, 10 mM EDTA, 10 mM EGTA, 15 mM β-mercaptoethanol, and 0.1 mM PMSF (Russell and Storey 1995). The homogenate was sonicated for 3 cycles of 30 s ON and 30 s OFF using Q500 sonicator (Qsonica) for proper breakage of mitochondria. The homogenate was then centrifuged at 10,000 *g* for 10 min followed by 14,000 *g* for 30 min at 4 °C, and the resultant supernatant was used for the enzyme assays.

To determine the activities of hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40), a 10% tissue homogenate was also prepared in a homogenizing buffer containing 50 mM Tris–HCl buffer (pH 7.4), 0.3 M Sucrose, 1 mM EDTA, 2 mM $MgCl_2$ and 2 mM β-mercaptoethanol. The homogenate was then treated with 0.5% Triton X-100 (1:1) for 30 min, followed by sonication for 3 cycles of 30 s ON and 30 s OFF using Q500 sonicator (Qsonica). The homogenate was then centrifuged at

10,000 *g* for 10 min followed by 14,000 *g* for 30 min, and the resultant supernatant was used for the enzyme assays.

Assay of enzyme activities

The activities of enzymes were assayed spectrophotometrically at 340 nm. In brief, 1 ml of reaction mixture was preincubated at 25 °C for 5 min. The tissue extract was added to the preincubated reaction mixture in order to start the reaction. OD was recorded at 340 nm at 30 s interval for 10 min and the change in OD values (ΔE) was taken for calculating the enzyme activity using 6.22×10^6 as molar extinction coefficient value for NADH/NADPH. One unit of enzyme activity was expressed as that amount of enzyme, which catalyzed the oxidation or reduction of 1 µmol of coenzymes ($NADP^+$, NADH and NADPH) per min at 25 °C. The specific activity of the enzymes was expressed as the units of enzyme activity per mg protein.

The reaction mixture for GPase assay contained 60 µmol potassium phosphate buffer (pH 7.2), 50 µmol $NADP^+$, 5 µmol glucose 1,6-bisphosphate, 2.5 µmol AMP, 5 units phosphoglucomutase, 5 units G6PDH, 10 mg glycogen, and 50 µl tissue extract. The reaction mixture also contained 10 µmol caffeine to determine the activity of GPase *a* alone (Moon et al. 1989). For GSase assay, the reaction mixture contained 60 µmol Imidazole–HCl buffer (pH 7.2), 5 µmol phosphoenolpyruvate, 6 µmol UDP-glucose, 0.15 µmol NADH, 150 µmol KCl, 15 µmol $MgCl_2$, 2 mg glycogen, 10 units pyruvate kinase, 10 units lactate dehydrogenase, and 50 µl tissue extract. The reaction mixture did not contain 5 µmol glucose 6-phosphate while measuring the GSase *a* activity alone (Passonneau and Rottenberg 1973).

The activity of hexokinase was assayed following the method as described by Bergmeyer (1974). 1 ml of reaction mixture contained 50 µmol Tris–HCl buffer (pH 7.4), 5 µmol D-glucose, 0.2 µmol $NADP^+$, 0.9 µmol ATP, 5 µmol $MgCl_2$, 10 units G6PDH, and 50 µl tissue extract. The activity of phosphofructokinase was assayed following the method as described by Buckwitz et al. (1988). 1 ml of reaction mixture contained 80 µmol Tris–HCl (pH 7.2), 5 µmol fructose 6-phosphate, 0.2 µmol NADPH, 0.8 µmol ATP, 0.9 µmol $MgCl_2$, 90 µmol KCl, 1 µmol K_2HPO_4, 8.0 µg aldolase, 3.3 µg triose phosphate isomerase, 3.3 µg glycerophosphate dehydrogenase and 50 µl tissue extract. The activity of pyruvate kinase was assayed following the method as described by Bücher and Pfleiderer (1955). 1 ml of reaction mixture contained 50 µmol Imidazole–HCl buffer (pH 7.6), 5 µmol phosphoenolpyruvate, 0.2 µmol NADH, 1.5 µmol ADP, 120 µmol KCl, 30 µmol $MgSO_4$, 10 units lactate dehydrogenase, and 50 µl tissue extract.

Statistical analysis

The data were obtained from at least three different experiments and statistically analyzed and presented as mean ± SEM. Mean values of the control and the treated samples were then compared using one way ANOVA using Statistical Package for the Social Sciences 17.0 software by Turkey post-hoc analyses with $p < 0.05$ regarded as statistically significant.

Results

Acute toxicity test

The acute toxicity test was performed in order to evaluate the toxicity of As and Pb on *H. fossilis*. The percentage mortality of *H. fossilis* during the exposure time at 48 h, 72 h and 96 h, respectively, was determined and indicated in Fig. 1 (A, B), which showed that the mortality of the fish was increased with increasing As_2O_3 and $PbCl_2$ concentration. The 96 h LC_{50} values of As_2O_3 were calculated and was found to be 35.097 ppm; whereas, that of $PbCl_2$ was 66.205 ppm. Based on these findings, the 96 h LC_{50} values of As_2O_3 and $PbCl_2$ were taken for acute study; whereas, the LC_{50}/20th ppm values were taken for chronic study for estimating the glycogen content and the activities of selected enzymes as mentioned before involved in glycogen metabolism and glycolysis.

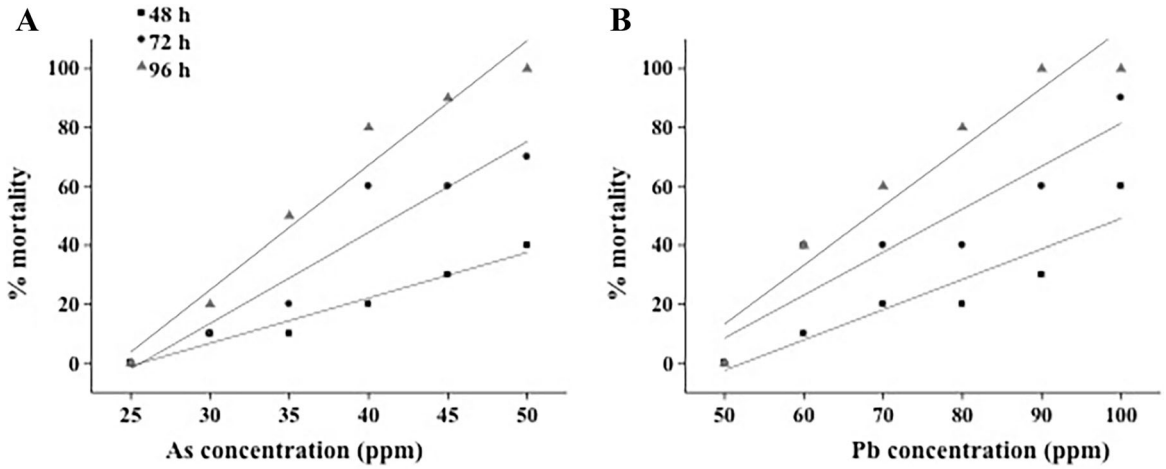

Fig. 1 Determination of 48 h, 72 h, and 96 h LC_{50} values of *H. fossilis* exposed to As_2O_3 (**a**) and $PbCl_2$ (**b**)

Estimation of glycogen

The glycogen content in liver and muscle tissues of *H. fossilis* was estimated for both acute and chronic treatment of As_2O_3 and $PbCl_2$ and presented in Table 1. Following the acute treatment of both As_2O_3 and $PbCl_2$, the glycogen content showed a gradual decrease in both liver and muscle tissues of *H. fossilis*. There was a significant decrease in the glycogen level (approximately 50–60%) within 96 h of As_2O_3 and $PbCl_2$ acute treatment as compared to the control. However, upon chronic treatment of As_2O_3 and $PbCl_2$, the glycogen content decreased up to 30–50% till 20 days, then there was a sudden increase in the glycogen level on 30 days. In corroboration to biochemical analysis of glycogen content, TEM studies were performed to find out the changes in the glycogen content in both liver and muscle tissues of *H. fossilis* exposed to 96 h LC_{50} values of As_2O_3 and $PbCl_2$. TEM studies of liver and muscle tissues of *H. fossilis* showed that the glycogen granules were decreased when exposed to an acute concentration of As_2O_3 and $PbCl_2$. Along with the decrease in glycogen level in the liver and muscle tissues of the catfish, the liver cells also showed a degenerated cell membrane, broken endoplasmic reticulum, disoriented nucleus, and dense mitochondria (Fig. 2a–c). Similarly, broken myofibrils were also observed in the muscle cells of the treated catfish exposed to heavy metals (Figs. 2d–f).

Effect of As and Pb on the activities of glycogen metabolic enzymes

The specific activity of GPase $a + b$ in the liver of *H. fossilis* did not show any significant changes on acute and chronic treatment of As_2O_3 and $PbCl_2$ (Table 2). However, the specific activity of GPase a in liver was increased by 61.54% and 53.85% upon acute treatment with As_2O_3 and $PbCl_2$, respectively; whereas, upon chronic treatment with As_2O_3 and $PbCl_2$, the specific activity of GPase a in liver of *H. fossilis* was increased by 56.25% and 50.00%, respectively, after 20 days. But, after 30 days of treatment, the specific activity of GPase a in liver of *H. fossilis* did not show any significant changes (Table 2). The specific activity of GSase $a + b$ in liver of *H. fossilis* also did not show any significant changes upon acute and chronic treatment with As_2O_3 and PbCl (Table 2). However, upon acute treatment of As_2O_3 and $PbCl_2$, the specific activity of GSase a was decreased by 70.00% and 57.50%, respectively; however, upon chronic treatment of As_2O_3 and $PbCl_2$, the specific activity of GSase a was decreased by 47.27% and 50.91%, respectively, after 20 days, but it showed slightly recovery after 30 days (Table 2).

The specific activity of GPase $a + b$ in the muscle of *H. fossilis* also did not show any significant changes (Table 3). Upon acute treatment with As_2O_3 and $PbCl_2$, the specific activity of GPase a in the muscle of *H. fossilis* was increased by 66.67% and 75.00%, respectively. However, upon chronic treatment of As_2O_3 and $PbCl_2$, the specific activity of GPase a in the muscle of *H. fossilis* was also increased by 81.82% and 72.73%, respectively, on 20 days; however, after 30 days of treatment, the specific activity GPase a was recovered and

Table 1 Evaluation of glycogen content in liver and muscle of *H. fossilis* upon acute (35.09 ppm of As_2O_3; 66.20 ppm of $PbCl_2$) and chronic (LC_{50}/20th ppm of both As_2O_3 and $PbCl_2$) exposure of As_2O_3 and $PbCl_2$

Acute treatment						
	24 h			48 h		
	Control	As treated	Pb treated	Control	As treated	Pb treated
Liver	13.29 ± 0.32	14.81 ± 1.56 (+11.44)	9.58 ± 1.47 (− 27.92)	13.22 ± 0.19	11.63 ± 0.97 (− 12.03)	8.02 ± 0.69[a] (− 39.33)
Muscle	0.99 ± 0.20	0.55 ± 0.06[a] (− 44.44)	0.68 ± 0.08[a] (− 31.31)	0.94 ± 0.20	0.67 ± 0.15 (− 28.72)	0.62 ± 0.16[a] (− 34.04)
Acute treatment						
	72 h			96 h		
	Control	As treated	Pb treated	Control	As treated	Pb treated
Liver	13.03 ± 0.12	10.10 ± 0.88 (− 22.49)	6.30 ± 0.50[a] (− 51.65)	12.88 ± 0.43	5.95 ± 0.24[a] (− 53.80)	5.04 ± 1.19[a] (− 60.87)
Muscle	0.97 ± 0.04	0.59 ± 0.15[a] (− 39.18)	0.47 ± 0.08[a] (− 51.55)	0.83 ± 0.05	0.33 ± 0.06[a] (− 60.24)	0.37 ± 0.07[a] (− 55.42)
Chronic treatment						
	10 days			20 days		
	Control	As treated	Pb treated	Control	As treated	Pb treated
Liver	13.74 ± 0.30	10.73 ± 0.21 (− 21.91)	10.07 ± 1.41[a] (− 26.71)	12.36 ± 0.42	8.92 ± 2.94 (− 27.83)	7.79 ± 2.32[a] (− 36.97)
Muscle	0.84 ± 0.01	0.66 ± 0.04 (− 21.43)	0.58 ± 0.02[a] (− 30.95)	0.80 ± 0.01	0.58 ± 0.04 (− 27.50)	0.43 ± 0.04[a] (− 46.25)
Chronic treatment						
	30 days					
	Control		As treated		Pb treated	
Liver	11.60 ± 0.72		13.26 ± 1.20 (+14.31)		14.60 ± 2.75 (+25.86)	
Muscle	0.78 ± 0.02		0.85 ± 0.15 (+8.97)		1.11 ± 0.26[a] (+42.31)	

Values are expressed as mean ± S.E.M. (n =3)

Percentage of decrease (−) or increase (+) in glycogen content of the fish exposed to As_2O_3 and $PbCl_2$ with respect to their controls is given in parentheses

[a]P value significant at < 0.05 in As and Pb treated fish with respect to their controls

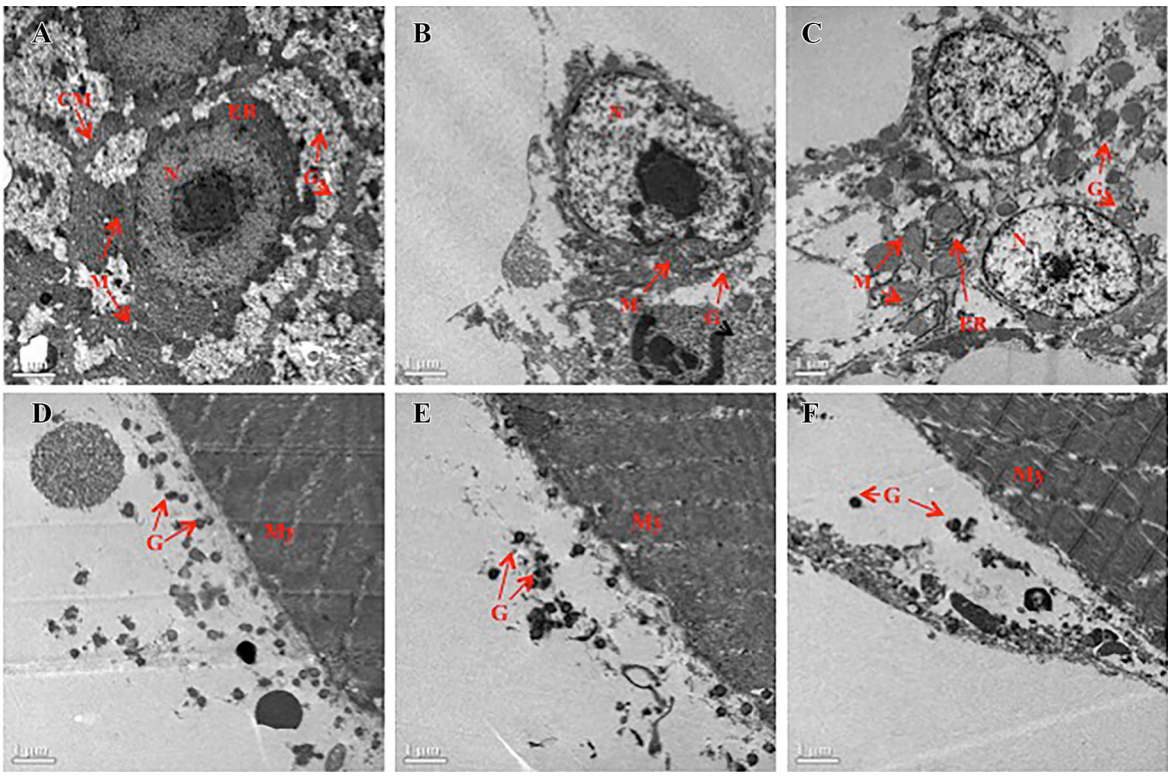

Fig. 2 Effect of As_2O_3 and $PbCl_2$ on glycogen particles in liver (**a**–**c**) and muscle (**d**–**f**) tissues of *H. fossilis*. The catfish was treated with LC_{50} values of As_2O_3 and $PbCl_2$ and glycogen particles were observed using transmission electron microscopy. **a** Control; **b** exposed to 35.09 ppm As_2O_3 for ~ 96 h; **c** exposed to 66.20 $PbCl_2$ for ~ 96 h; **d** control; **e** exposed to 35.09 ppm As_2O_3 for ~ 96 h; **f** exposed to 66.20 $PbCl_2$ for ~ 96 h. *CM* cell membrane, *ER* endoplasmic reticulum, *G* glycogen granules, *M* mitochondria, *My* myofibrils, *N* nucleus

showed no significant changes when compared to the control (Table 3). In the muscle of *H. fossilis*, the specific activity of GSase $a + b$ did not show any significant changes upon acute and chronic treatment with As_2O_3 and $PbCl_2$ (Table 3). The specific activity of GSase *a* in the muscle of *H. fossilis* was decreased by 57.58% and 53.03% upon acute treatment of As_2O_3 and $PbCl_2$, respectively; however, upon chronic treatment with As_2O_3 and $PbCl_2$, the specific activity of GSase *a* in the muscle of *H. fossilis* was decreased by 47.76% and 52.24%, respectively, up to 20 days (Table 3).

Effect of As and Pb on activities of selected glycolytic enzymes

The effect of As and Pb was also tested on the activities of selected glycolytic enzymes. The specific activity of hexokinase in the liver of *H. fossilis* was decreased by 69.70% and 59.09% upon acute exposure with As_2O_3 and $PbCl_2$, respectively; however, upon chronic treatment, the specific activity of hexokinase in the liver of *H. fossilis* was decreased gradually up to 62.07% and 60.34%, respectively (Table 4). The specific activity of phosphofructokinase in the liver of *H. fossilis* did not show any significant changes when treated with both acute and chronic concentration of As_2O_3 and $PbCl_2$ (Table 4). The specific activity of pyruvate kinase in the liver of *H. fossilis* was decreased by 51.12% and 65.59% upon acute treatment with As_2O_3 and $PbCl_2$, respectively. On chronic treatment with As_2O_3 and $PbCl_2$, the specific activity of pyruvate kinase in the liver of *H. fossilis* was also found to be decreased gradually up to 49.81% and 46.41%, respectively (Table 4).

The specific activity of hexokinase in the muscle of *H. fossilis* was decreased by 50.00% and 55.26% on acute treatment of As_2O_3 and $PbCl_2$, respectively. However, upon chronic treatment of As_2O_3 and $PbCl_2$, the specific activity of hexokinase was decreased gradually up to 44.83% and 41.38%, respectively (Table 5). The specific activity of phosphofructokinase in the muscle of *H. fossilis*, showed no significant changes on both acute and chronic treatment of As_2O_3 and $PbCl_2$ (Table 5). However, in the muscle of *H. fossilis*, the specific

Table 2 Effects of acute and chronic concentrations of As_2O_3 and $PbCl_2$ on the specific activity (units/mg protein) of GPase ($a + b$), GPase (a), GSase ($a + b$) and GSase (a) enzymes in liver tissue of *H. fossilis*

Acute treatment	GPase ($a + b$)		GPase (a)	
Treatment (ppm)	48 h	96 h	48 h	96 h
Control	0.017 ± 0.002	0.016 ± 0.001	0.014 ± 0.002	0.013 ± 0.001
As_2O_3 (35.09)	0.021 ± 0.001 (+21.15)	0.018 ± 0.002 (+10.20)	0.018 ± 0.002 (+28.57)	0.021 ± 0.004[a] (+61.54)
$PbCl_2$ (66.20)	0.020 ± 0.001 (+15.39)	0.017 ± 0.001 (+4.08)	0.017 ± 0.001 (+21.43)	0.020 ± 0.002[a] (+53.85)
Acute treatment	GSase ($a + b$)		GSase (a)	
Treatment (ppm)	48 h	96 h	48 h	96 h
Control	0.039 ± 0.005	0.038 ± 0.002	0.038 ± 0.002	0.040 ± 0.002
As_2O_3 (35.09)	0.035 ± 0.003 (− 10.26)	0.037 ± 0.003 (− 2.63)	0.021 ± 0.003[a] (− 44.74)	0.012 ± 0.002[a] (− 70.00)
$PbCl_2$ (66.20)	0.0036 ± 0.003 (− 7.69)	0.039 ± 0.001 (+2.63)	0.032 ± 0.004 (− 15.79)	0.017 ± 0.003[a] (− 57.50)

Chronic treatment	GPase ($a + b$)			GPase (a)		
Treatment (ppm)	10 days	20 days	30 days	10 days	20 days	30 days
Control	0.018 ± 0.001	0.016 ± 0.001	0.017 ± 0.001	0.015 ± 0.002	0.016 ± 0.003	0.014 ± 0.001
As_2O_3 (1.75)	0.022 ± 0.002 (+22.22)	0.019 ± 0.001 (+18.75)	0.018 ± 0.001 (+5.88)	0.017 ± 0.001 (+13.33)	0.025 ± 0.003[a] (+56.25)	0.016 ± 0.003 (+11.63)
$PbCl_2$ (3.31)	0.020 ± 0.001 (+11.11)	0.017 ± 0.002 (+6.25)	0.019 ± 0.001 (+11.76)	0.018 ± 0.001 (+20.00)	0.024 ± 0.002[a] (+50.00)	0.015 ± 0.002 (+4.65)
Chronic treatment	GSase ($a + b$)			GSase (a)		
Treatment (ppm)	10 days	20 days	30 days	10 days	20 days	30 days
Control	0.038 ± 0.004	0.037 ± 0.003	0.039 ± 0.005	0.052 ± 0.003	0.055 ± 0.003	0.054 ± 0.007
As_2O_3 (1.75)	0.029 ± 0.003 (− 23.68)	0.030 ± 0.002 (− 18.92)	0.037 ± 0.003 (− 5.13)	0.041 ± 0.002 (− 21.15)	0.029 ± 0.003[a] (− 47.27)	0.048 ± 0.002 (− 11.11)
$PbCl_2$ (3.31)	0.034 ± 0.001 (− 10.53)	0.032 ± 0.004 (− 13.51)	0.031 ± 0.002 (− 20.51)	0.037 ± 0.002 (− 28.85)	0.027 ± 0.002[a] (− 50.91)	0.049 ± 0.005 (− 9.26)

Values are expressed as mean ± S.E.M. (n =3)

Percentage of decrease (−) or increase (+) in specific activity of the fish exposed to As_2O_3 and $PbCl_2$ with respect to their controls is given in parentheses

[a]P value significant at < 0.05 in As and Pb treated fish with respect to their controls. One unit of enzyme activity is defined as 1 μmol of $NADP^+$ reduced in case of GPase or NADH oxidized in case of GSase per minute at 25 °C. Specific activity of the enzymes is expressed as the units of enzyme activity per mg protein

Table 3 Effects of acute and chronic concentrations of As_2O_3 and $PbCl_2$ on the specific activity (units/mg protein) of GPase ($a + b$), GPase (a), GSase ($a + b$) and GSase (a) enzymes in muscle tissue of *H. fossilis*

Acute treatment	GPase ($a + b$)		GPase (a)	
Treatment (ppm)	48 h	96 h	48 h	96 h
Control	0.015 ± 0.004	0.017 ± 0.001	0.013 ± 0.001	0.012 ± 0.001
As_2O_3 (35.09)	0.013 ± 0.001 (− 15.22)	0.019 ± 0.002 (+11.76)	0.015 ± 0.001 (+15.38)	0.018 ± 0.001[a] (+66.67)
$PbCl_2$ (66.20)	0.016 ± 0.005 (+4.35)	0.021 ± 0.001 (+23.53)	0.017 ± 0.002[a] (+30.77)	0.021 ± 0.001[a] (+75.00)
Acute treatment	GSase ($a + b$)		GSase (a)	
Treatment (ppm)	48 h	96 h	48 h	96 h
Control	0.065 ± 0.002	0.064 ± 0.003	0.067 ± 0.003	0.066 ± 0.004
As_2O_3 (35.09)	0.060 ± 0.001 (− 7.69)	0.059 ± 0.001 (− 7.81)	0.044 ± 0.005[a] (− 34.33)	0.028 ± 0.004[a] (− 57.58)
$PbCl_2$ (66.20)	0.063 ± 0.002 (− 13.85)	0.061 ± 0.001 (− 10.94)	0.054 ± 0.008 (− 19.40)	0.031 ± 0.005[a] (− 53.03)

Chronic treatment	GPase ($a + b$)			GPase (a)		
Treatment (ppm)	10 days	20 days	30 days	10 days	20 days	30 days
Control	0.016 ± 0.001	0.015 ± 0.001	0.017 ± 0.001	0.012 ± 0.002	0.011 ± 0.001	0.010 ± 0.001
As_2O_3 (1.75)	0.018 ± 0.002 (+12.50)	0.019 ± 0.001 (+26.67)	0.018 ± 0.002 (+5.88)	0.014 ± 0.001 (+16.67)	0.020 ± 0.002[a] (+81.82)	0.012 ± 0.001 (+20.00)
$PbCl_2$ (3.31)	0.019 ± 0.002 (+18.75)	0.016 ± 0.001 (+ 6.67)	0.020 ± 0.002 (+17.65)	0.015 ± 0.001 (+25.00)	0.019 ± 0.002[a] (+72.73)	0.011 ± 0.001 (+10.00)
Chronic treatment	GSase ($a + b$)			GSase (a)		
Treatment (ppm)	10 days	20 days	30 days	10 days	20 days	30 days
Control	0.065 ± 0.002	0.064 ± 0.002	0.069 ± 0.006	0.064 ± 0.004	0.067 ± 0.001	0.068 ± 0.003
As_2O_3 (1.75)	0.062 ± 0.003 (− 4.61)	0.054 ± 0.001 (− 15.62)	0.061 ± 0.002 (− 11.59)	0.053 ± 0.003 (− 17.19)	0.035 ± 0.004[a] (− 47.76)	0.057 ± 0.003 (− 16.18)
$PbCl_2$ (3.31)	0.057 ± 0.002 (− 12.31)	0.060 ± 0.002 (− 6.25)	0.059 ± 0.005 (− 14.49)	0.047 ± 0.003 (− 26.56)	0.032 ± 0.004[a] (− 52.24)	0.059 ± 0.002 (− 13.23)

Values are expressed as mean ± S.E.M. (n =3)

Percentage of decrease (−) or increase (+) in specific activity of the fish exposed to As_2O_3 and $PbCl_2$ with respect to their controls is given in parentheses

[a]P value significant at < 0.05 in As and Pb treated fish with respect to their controls. One unit of enzyme activity is defined as 1 μmol of $NADP^+$ reduced in case of GPase or NADH oxidized in case of GSase per minute at 25 °C. Specific activity of the enzymes is expressed as the units of enzyme activity per mg protein

Table 4 Effects of acute and chronic concentrations of As_2O_3 and $PbCl_2$ on the specific activity (units/mg protein) of hexokinase, phosphofructokinase and pyruvate kinase enzymes in liver tissue of *H. fossilis*

	Hexokinase		Phosphofructokinase	
Acute treatment Treatment (ppm)	48 h	96 h	48 h	96 h
Control	0.067 ± 0.003	0.066 ± 0.004	0.040 ± 0.002	0.041 ± 0.002
As_2O_3 (35.09)	0.042 ± 0.004[a] (− 37.31)	0.020 ± 0.001[a] (− 69.70)	0.034 ± 0.004 (− 15.00)	0.036 ± 0.001 (− 12.19)
$PbCl_2$ (66.20)	0.048 ± 0.001 (− 28.36)	0.027 ± 0.001[a] (− 59.09)	0.038 ± 0.003 (− 5.00)	0.040 ± 0.002 (− 2.44)

	Pyruvate kinase	
Acute treatment Treatment (ppm)	48 h	96 h
Control	0.337 ± 0.033	0.311 ± 0.006
As_2O_3 (35.09)	0.173 ± 0.004[a] (− 48.66)	0.152 ± 0.012[a] (− 51.12)
$PbCl_2$ (66.20)	0.140 ± 0.042[a] (− 58.46)	0.107 ± 0.019[a] (− 65.59)

	Hexokinase			Phosphofructokinase		
Chronic treatment Treatment (ppm)	10 days	20 days	30 days	10 days	20 days	30 days
Control	0.061 ± 0.003	0.059 ± 0.003	0.058 ± 0.005	0.046 ± 0.006	0.047 ± 0.004	0.045 ± 0.003
As_2O_3 (1.75)	0.053 ± 0.003 (− 13.11)	0.039 ± 0.003[a] (− 33.90)	0.022 ± 0.003[a] (− 62.07)	0.038 ± 0.002 (− 17.39)	0.042 ± 0.005 (− 10.64)	0.044 ± 0.005 (− 2.22)
$PbCl_2$ (3.31)	0.048 ± 0.002 (− 21.31)	0.029 ± 0.003[a] (− 50.85)	0.023 ± 0.002[a] (− 60.34)	0.041 ± 0.001 (− 10.87)	0.037 ± 0.003 (− 21.28)	0.039 ± 0.004 (− 13.33)

	Pyruvate kinase		
Chronic treatment Treatment (ppm)	10 days	20 days	30 days
Control	0.271 ± 0.027	0.241 ± 0.008	0.265 ± 0.016
As_2O_3 (1.75)	0.213 ± 0.018 (− 21.40)	0.154 ± 0.030[a] (− 36.10)	0.133 ± 0.020[a] (− 49.81)
$PbCl_2$ (3.31)	0.253 ± 0.055 (− 6.64)	0.177 ± 0.032 (− 26.56)	0.142 ± 0.006[a] (− 46.41)

Values are expressed as mean ± S.E.M. (n =3)

Percentage of decrease (−) or increase (+) in specific activity of the fish exposed to As_2O_3 and $PbCl_2$ with respect to their controls is given in parentheses

[a]P value significant at < 0.05 in As and Pb treated fish with respect to their controls. One unit of enzyme activity is defined as 1 μmol of $NADP^+$ reduced in case of hexokinase or NADH oxidized in case of phosphofructokinase and pyruvate kinase per minute at 25 °C. Specific activity of the enzymes is expressed as the units of enzyme activity per mg protein

Table 5 Effects of acute and chronic concentrations of As_2O_3 and $PbCl_2$ on the specific activity (units/mg protein) of hexokinase, phosphofructokinase and pyruvate kinase enzymes in muscle tissue of *H. fossilis*

	Hexokinase		Phosphofructokinase	
Acute treatment Treatment (ppm)	48 h	96 h	48 h	96 h
Control	0.039 ± 0.001	0.038 ± 0.002	0.032 ± 0.003	0.034 ± 0.002
As_2O_3 (35.09)	0.030 ± 0.001 (− 23.08)	0.019 ± 0.002[a] (− 50.00)	0.029 ± 0.002 (− 9.37)	0.027 ± 0.002 (− 20.59)
$PbCl_2$ (66.20)	0.025 ± 0.001[a] (− 35.90)	0.017 ± 0.003[a] (− 55.26)	0.031 ± 0.002 (− 3.12)	0.030 ± 0.004 (− 11.76)

	Pyruvate kinase	
Acute treatment Treatment (ppm)	48 h	96 h
Control	3.183 ± 0.445	3.321 ± 0.234
As_2O_3 (35.09)	2.685 ± 0.358 (− 15.65)	2.271 ± 0.075[a] (v31.62)
$PbCl_2$ (66.20)	2.120 ± 0.449[a] (− 33.40)	1.696 ± 0.408[a] (− 48.93)

	Hexokinase			Phosphofructokinase		
Chronic treatment Treatment (ppm)	10 days	20 days	30 days	10 days	20 days	30 days
Control	0.031 ± 0.004	0.030 ± 0.003	0.029 ± 0.005	0.035 ± 0.002	0.034 ± 0.004	0.036 ± 0.004
As_2O_3 (1.75)	0.024 ± 0.002 (− 22.58)	0.025 ± 0.002 (− 16.67)	0.016 ± 0.001[a] (− 44.83)	0.029 ± 0.002 (− 17.14)	0.031 ± 0.004 (− 8.82)	0.038 ± 0.005 (+5.56)
$PbCl_2$ (3.31)	0.022 ± 0.002 (− 29.03)	0.019 ± 0.003[a] (− 36.67)	0.017 ± 0.002[a] (− 41.38)	0.032 ± 0.005 (− 8.57)	0.028 ± 0.004 (− 17.65)	0.031 ± 0.003 (− 13.89)

	Pyruvate kinase		
Chronic treatment Treatment (ppm)	10 days	20 days	30 days
Control	2.630 ± 0.206	2.520 ± 0.065	2.761 ± 0.052
As_2O_3 (1.75)	2.002 ± 0.066 (− 23.88)	1.796 ± 0.100 (− 28.73)	0.864 ± 0.121[a] (− 68.71)
$PbCl_2$ (3.31)	2.024 ± 0.181 (− 23.04)	1.294 ± 0.199[a] (− 48.65)	0.610 ± 0.036[a] (− 77.91)

Values are expressed as mean ± S.E.M. (n =3)

Percentage of decrease (−) or increase (+) in specific activity of the fish exposed to As_2O_3 and $PbCl_2$ with respect to their controls is given in parentheses

[a]P value significant at < 0.05 in As and Pb treated fish with respect to their controls. One unit of enzyme activity is defined as 1 µmol of $NADP^+$ reduced in case of hexokinase or NADH oxidized in case of phosphofructokinase and pyruvate kinase per minute at 25 °C. Specific activity of the enzymes is expressed as the units of enzyme activity per mg protein

activity of pyruvate kinase was decreased by 31.62% and 48.93% upon acute treatment of As_2O_3 and $PbCl_2$, respectively; whereas, upon chronic treatment of As_2O_3 and $PbCl_2$, the specific activity of pyruvate kinase was observed to be decreased gradually up to 68.71% and 77.91%, respectively (Table 5).

Discussion

Sensitivity of fish to As and Pb differs among different species of fishes. A possible explanation for these differences to trace metals might be explained as a function of metallothionein synthesis, which is believed to provide a protective role against toxic effect of metals in aquatic animals including fishes (Roesijadi 1992; Hollis et al. 2001). Acute toxicity tests were conducted to determine LC_{50} values of As_2O_3 and $PbCl_2$ on *H. fossilis* and it was found to be 35.097 ppm and 66.205 ppm, respectively. Earlier findings show that 96 h LC_{50} of As_2O_3 is 8.91 ppm for *Danio rerio* (Bhavani and Karuppasamy 2014), 24.19 ppm for *Chana punctatus* (Amsath et al. 2017), 49.5 ppm for *Poecilia sphenops* (Patel et al. 2017), and 18.21 ppm for *Anabas testudineus* (Akter et al. 2008). Previous studies also show that the 96 h LC_{50} of $PbCl_2$ is 1.01 ppm for *Anabas testudineus* (Parvin et al. 2011), 58.0 ppm for *Cyprinus carpio* (Hedayati et al. 2013) and 86.84 ppm for *Metynnis fasciatus* (Sadeghi and Imanpoor 2015).

In the present study, acute and chronic exposure of *H. fossilis* to As and Pb exhibited significant alterations in the glycogen content in the liver and muscle tissues of *H. fossilis.* Glycogen granules were shown to be depleted in the fish exposed to an acute concentration of heavy metals (As and Pb) as evidenced from the TEM studies. Depletion in glycogen granules in liver of zebra fish is also reported by Macirella et al. (2016) when treated with mercury chloride. The depletion of glycogen may be due to rapid turnover of the glycogen. Ananth et al. (2014) and Kavidha and Muthulingam (2014) have also reported the decline of glycogen content in grass carps, *Ctenopharyngodon idella* and *Cyprinus carpio* when exposed to As and Pb. Exposure to chronic concentrations of As and Pb showed that the glycogen content was decreased up to 20 days, but after 20 days there was an increase in glycogen content in both liver and muscle tissues of the fish. Similar trend has also been reported by Sastry and Subhadra (1982) in *H. fossilis* when exposed to cadmium. This may be due to the reduced rates of glycogenolysis resulting from the cumulative toxic effects of As and Pb.

The decreasing glycogen content in both liver and muscle tissues of the fish was resulted by the increasing specific activity of GPase *a*. Upon acute treatment of As and Pb, GPase *a* specific activity increases, which correspond with the breakdown of glycogen granules in both liver and muscle tissues of *Clarias batrachus* (Begum 2004). However, upon chronic exposure to As and Pb the specific activity of GPase *a* was increased after 20 days of treatment, and recovered after 30 days, which may be correlated with the rapid restoration of glycogen content in both the tissues. In the present study, the decrease in the specific activities of GSase *a* in liver and muscle tissues of *H. fossilis* may be correlated with the increase in glycogenolysis. Similar results are also observed when *Salmo salar* is exposed to cadmium (Soengas et al. 1996).

In this study, the specific activity of hexokinase was decreased when exposed to an acute concentrations of As and Pb. Carvalho and Fernandes (2008) show that hexokinase activity in *Prochilodus lineatus* decreases when exposed to copper. The specific activity of phosphofructokinase showed no significant changes when treated with As and Pb, which is similar to the effect of copper in *Salmo trutta* (Beaumont et al. 2000). Pyruvate kinase specific activity was found to decrease when exposed to acute and chronic concentrations of As and Pb. Previous studies also indicate that heavy metals such as copper inhibits pyruvate kinase specific activity in tropical fish, *Prochilodus lineatus* (Carvalho and Fernandes 2008).

Conclusions

The results of the present study indicated that As and Pb caused significant changes in carbohydrate metabolism in the fish and these alterations could be attributed to the toxic stress induced by these two heavy metals on the model fish.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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