

## STUDY OF THE EFFECT OF RESIDUAL CHLORINE ON SERUM IRON AND RELATED PARAMETERS

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### ABSTRACT

The major purpose of the present investigation was to study the effects of different concentrations of residual chlorine on serum iron related parameters in rats. Feeding male rats with water containing 80 ppm of residual chlorine daily for 5 days reduced serum levels of ceruloplasmin, copper, iron and hemoglobin by approximately 27%, 23%, 6% and 4%, respectively. Higher reductions in serum ceruloplasmin (35%), copper (50%), Fe (17%) and hemoglobin (14%) were observed when rats were given water containing 160 ppm of residual chlorine daily for 5 days. The total iron binding capacity (TIBC) level was elevated by 9 and/or 13 percent respectively. Daily feeding of rats for 10 days with water containing 160 ppm residual chlorine caused significant reductions in serum levels of ceruloplasmin (50%), copper (54%) iron (17%) and hemoglobin (17%). Serum TIBC was elevated by 25%. Long term effects of residual chlorine on the above parameters were also investigated. It can therefore be concluded that residual chlorine in drinking water may interfere with iron metabolism. The relationship between the occurrence of anemia and residual chlorine toxicity has been discussed.

**Keywords:** Residual chlorine, ceruloplasmin, hemoglobin, copper, iron, TIBC.

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### INTRODUCTION

The use of chlorine as a disinfectant for drinking water supplies has been probably the most important event in the entire history of water supply industry. Disinfection has virtually eliminated enteric diseases such as cholera, typhoid fever and various types of dysenteries throughout the world.<sup>1</sup> The first notion that water chlorination may result in some undesirable side effects was the observation of trihalomethanes (THMs) which were formed as by-products of disinfection.<sup>2</sup> Despite long and widespread use of chlorine as a disinfectant for drinking water, very little information is available on the effects of ingested chlorine on health.

Recently, it has been reported that the presence of residual chlorine interferes with a number of biochemical

events in the body. Chlorine does not appear to be directly toxic with the reported LD50 of 850 mg/kg (given as calcium hypochlorite) in the rat.<sup>3</sup>

In short-term studies, the liver and kidney appeared to be target organs. A single gavage dose of 143 mg/kg chlorine (as sodium hypochlorite) given to rats resulted in morphological and biochemical changes in the liver within a 2-day period.<sup>4</sup> Decreased thyroxine, increased plasma cholesterol, and increased heart weight have been reported in pigeons consuming 2 to 15 mg/l chlorine (as sodium hypochlorite) in drinking water for three months.<sup>5</sup> The increased plasma cholesterol level in pigeons suggests a potential cardiovascular effect from chlorine. Research is in progress in monkeys and humans to further evaluate this potential effect. Chlorinated water has also been implicated in bladder and colon cancer in humans,<sup>6</sup> but this effect is yet to be substan-

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tiated.

In the present project the short and long term effects of residual chlorine on serum iron and related parameters have been investigated. Previous reports from a number of laboratories have postulated that the binding of iron to serum apo-transferrin requires bicarbonate ion<sup>7</sup> and chlorine in the plasma may interfere with iron metabolism either through transferrin or other routes.

### MATERIALS AND METHODS

Male Wistar rats (180-200 g) were purchased from Pasteur Institute (Tehran, Iran). They were allowed access to food and water at standard conditions regarding light and room temperature. Animals (200-250 g) were fed with water containing different levels of residual chlorine as indicated. The control group received drinking water with no residual chlorine as checked by iodometric method<sup>8</sup> and the experimental group which received water containing different levels of residual chlorine. To prepare residual chlorine, a stock dosing solution was prepared by the addition of 5.25% sodium hypochlorite solution to distilled water, to a final concentration of 1 mg/ml chlorine. Aliquots of this solution were diluted to obtain the desired concentration of residual chlorine.

Following the completion of each experiment the animals were then killed by cervical dislocation at different time intervals. The sera were collected by centrifugation and used for determination of biochemical parameters listed below. Serum ceruloplasmin concentration was determined by the method of Kachmar and Moss (1976), which is based on enzyme oxidase activity using paraphenylenediamine hydrochloride as substrate and measuring the color produced at 525 nm.<sup>9</sup> Serum copper concentration was measured by atomic absorption spectrometry (Perkin-Elmer model 2380).<sup>10</sup>

Hemoglobin (Hb) was determined by the method of Fairbanks.<sup>11</sup> Serum iron and TIBC were determined using phenanthroline as a chromogen forming agent.<sup>12</sup> Due to the small surface of the bottles used for feeding the rats with water containing chlorine and also based on chlorine measurements at the beginning and at the end of each experiment, the amount of lost chlorine was insignificant.

### RESULTS

#### Short term effect of residual chlorine on serum iron parameters

In the first series of experiments, short term effects of residual chlorine on serum iron related parameters including ceruloplasmin, copper, iron, TIBC and hemoglobin were studied over a period of 5 and 10 days of residual chlorine feeding. Feeding rats with 80 ppm residual chlorine in drinking water daily for 5 days reduced serum levels of ceruloplasmin, copper iron and hemoglobin by 27%, 24% 6%, and 4% respectively in comparison to controls. When they were fed with 160 ppm residual chlorine daily for 5 days, further reductions in serum concentrations of ceruloplasmin (35%), copper (50%), iron (17%) and hemoglobin (6%) were observed. The TIBC level was elevated by 15%, (Table I).

The effect of a 10 day period of residual chlorine (80 and 160 ppm) feeding on the same parameters was evaluated. Data obtained are presented in Table II. Following 5 days of residual chlorine administration, significant reductions in serum ceruloplasmin (44%), copper (22%) iron (14%) and hemoglobin (14%) were observed.

When 160 ppm of residual chlorine was used instead of 80 ppm further reductions were observed in serum levels of ceruloplasmin (50%), copper (55%), iron (17%) and hemoglobin (17%) (Table II).

Table I. Effect of residual chlorine on serum iron related parameters in rats after 5 and 10 days.

Daily Treatment	Ceruloplasmin (mg/dl)	Cu (µg/dl)	Fe (µg/dl)	TIBC (µg/dl)	Hb (g/dl)
Control	43.5±3.5	73.1±4.3	243.5±9.8	390.1±7.1	13.9±0.4
80 ppm/5 days	31.9±2.1	55.9±5.5*	228.3±6.1*	425.7±6.1*	13.5±0.3
160 ppm/5 days	28.2±1.8*	36.6±3.8	200.1±11.0	441.9±12.1	13.1±0.05
80 ppm/10 days	24.5±2.9*	56.9±3.2	210.0±12.1	431.5±10.7	12.1±0.3*
160 ppm/10 days	21.7±3.1*	32.8±4.1*	202.2±15.9	489.6±20.7	11.67±0.3*

mean±SE of 5 separate experiments.

\*Statistically significant at P<0.05.

Table II. Effect of residual chlorine on serum iron related parameters in rats after 22 and 44 days.

Daily Treatment	Ceruloplasmin (mg/dl)	Cu ( $\mu$ g/dl)	Fe ( $\mu$ g/dl)	TIBC ( $\mu$ g/dl)	Hb (g/dl)
Control	41.9 $\pm$ 1.7	67.1 $\pm$ 3.7	266.5 $\pm$ 15.7	382.4 $\pm$ 15.0	13.45 $\pm$ 0.2
10 ppm/22 days	41.9 $\pm$ 0.4	53.2 $\pm$ 1.6*	260.4 $\pm$ 3.7	431.2 $\pm$ 11.4*	13.2 $\pm$ 0.1
40 ppm/22 days	33.9 $\pm$ 1.8*	35.9 $\pm$ 3.4*	251.2 $\pm$ 1.2	509.2 $\pm$ 25.1*	12.5 $\pm$ 0.2*
10 ppm/44 days	40.0 $\pm$ 0.8*	52.0 $\pm$ 2.3*	180.0 $\pm$ 13.9*	383.0 $\pm$ 8.8	12.0 $\pm$ 0.3*
40 ppm/44 days	28.8 $\pm$ 3.0*	33.8 $\pm$ 3.5*	127.2 $\pm$ 11.9*	426.4 $\pm$ 46	11.7 $\pm$ 0.3*

Data are expressed as mean $\pm$ SE of five separate experiments.

\*Statistically significant at P<0.05.

Table III. Effect of residual chlorine on serum iron related parameters in rats after 66 and 88 days.

Daily Treatment	Ceruloplasmin (mg/dl)	Cu ( $\mu$ g/dl)	Fe ( $\mu$ g/dl)	TIBC ( $\mu$ g/dl)	Hb (g/dl)
Control	42.83 $\pm$ 2.1	64.1 $\pm$ 4.9	303.8 $\pm$ 22.06	375.8 $\pm$ 13.5	13.0 $\pm$ 0.0
20 ppm/66 days	28.2 $\pm$ 3.9*	41.0 $\pm$ 5.1	193.0 $\pm$ 12.6*	404.8 $\pm$ 3.6*	12.3 $\pm$ 0.3
20 ppm/88 days	27.8 $\pm$ 1.7*	32.0 $\pm$ 4.5*	148.5 $\pm$ 13.5*	424.2 $\pm$ 9.5*	11.8 $\pm$ 0.3*

Data are expressed as mean $\pm$ SE of five separate experiments.

\*Statistically significant at P<0.05.

### Long term effects of residual chlorine on serum iron related parameters

Further studies were carried out to determine the effects of residual chlorine on the levels of serum iron related parameters. Rats were fed daily with varying concentrations of residual chlorine (10-40 ppm) for 22-28 days. Results are presented in Table III.

## DISCUSSION

Addition of chlorine to water supply is a routine procedure for water disinfection throughout the world.<sup>13</sup> Residual chlorine in water enters the circulation via the gastrointestinal tract. In addition, it may enter the circulation via dialysis fluid in chronic renal failure patients who are undergoing hemodialysis. Residual chlorine from either source may interfere with a number of biochemical reactions and produce a number of pathophysiological disturbances such as disorders in lipid metabolism<sup>5</sup> and cancer.<sup>14</sup>

It has been reported that residual chlorine in water may damage the hematopoietic system and cause anemia.<sup>15</sup> The

exact mechanism by which residual chlorine interferes with iron metabolism is still a subject of speculation. Data presented in this paper show that administration of residual chlorine to the rat reduces serum levels of ceruloplasmin, copper, iron and hemoglobin significantly. Iron enters the circulation through intestinal mucosal cells undergoing a number of oxidation-reduction reactions.<sup>16,17</sup> In the blood, iron which is in ferric form binds to serum apotransferrin, the major iron carrier glycoprotein.<sup>18</sup> Following residual chlorine administration, the serum iron concentration is reduced and subsequently TIBC is increased. This supports the idea that residual chlorine interferes with the binding of iron to apotransferrin, leading to an increase of iron binding sites on the protein. The liver is the major site of apotransferrin and ceruloplasmin production,<sup>19</sup> thus damage to this organ may lead to reduced synthesis of apotransferrin and decreased ceruloplasmin levels. Ceruloplasmin is an  $\alpha_2$ -glycoprotein needed for the oxidation of Fe (II) to Fe (III) in the cell. The significant reduction in the level of this copper containing protein may disturb the oxidation of Fe (II) in the cell and interfere with iron metabolism.

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Significant reduction in serum ceruloplasmin levels following the administration of residual chlorine may be either due to reduction in liver ability to produce this glycoprotein or to prevention of the incorporation of copper into apoceruloplasmin in the liver.

Our study showed that residual chlorine administration decreases hemoglobin concentrations. Heme synthesis occurs in the mitochondria<sup>20,21</sup> of these cells, thus the elevation of the total iron binding sites of apotransferrin (TIBC) or the reduction in serum iron concentration cannot provide the needed iron for heme synthesis. It might be concluded that, residual chlorine in drinking water might interfere with iron metabolism and damage the hematopoietic system as already reported.<sup>15</sup> However further studies are needed to elucidate the molecular mechanisms of this interaction.

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