

EFFECT OF CHLORINATED WATER AND IT'S DISINFECTION BY-PRODUCTS (DBPS) IN SOME HYGIENIC AND PHYSIOLOGICAL TRAITS OF MALE RABBITS

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ABSTRACT

The study was performed to find out the effect of chlorine and disinfection by-products(DBPs) of chlorinated water in some hygienic and Physiological traits of male rabbits, this experiment was accomplished in animal research farm / Veterinary Medicine College, Baghdad University from 20th November 2017 up to 20th January 2018. Twenty-eight male rabbits were used at age of 3 month and randomly distributed equally in four groups(seven male) per group, body weight was considered as the following: First group(chlorine free water) as a control group, second group (tap water), third group(chlorine 3ppm), fourth group(chlorine 5ppm).All groups were daily fed on concentrated diet(pellet 75gm/ head), Alfalfa and specific water for each group were offered freely. Blood samples were collected and serum samples were obtained at biweekly intervals to measurement the hemoglobin concentration(Hb), Packed Cell Volume(PCV), malondialdehyde(MDA), glutathione, total protein, transaminase enzyme(AST,ALT), alkaline phosphate, bilirubin to detect hygienic and Physiological traits. Data were statistically analyzed, the results of this study revealed the significant ($P < 0.05$) and nonsignificant differences in some hygienic and Physiological traits among groups of different periods. Accordingly, it could be concluded from this study the effect of chlorine and it's disinfection by-products(DBPs) of chlorinated water lead to many changes in some hygienic and Physiological traits in male rabbits compare with tap water , so that water quality had direct effect on hygienic and productive performance of the animals.

INTRODUCTION

The water is considered an a necessary nutrient to fulfill many vital functions and productivity performance, likewise which involved in all fundamental physiological processes that occurring in the animal body and is a midst in which all reactions and transmission of nutrients, hormones, waste products, also support in motion of food meanwhile the gastrointestinal tract, too is a prime ingredient of body fluids systems^[1,2,3]. The substantial component of ecosystem is a water, it's preferable quality qualified by physical, chemical and biological characteristic, also it's very remarkable at compare to other nutrients, on account of used in considerable great quantities, thus water availability and quality are so important to animals validity and productivity ^[4, 5, 6]. Abbreviate water availability to livestock will minimize production rapidly and badly, also bad quality drinking water predominatingly a factor restrict intake, considering that water is consumed in large quantities, if the quality is bad there is an rise risk that water contaminants could reach a level that may be deleterious^[4,7,8], therefore water for livestock should be analyzed periodically^[6], Ruling to promote bad quality fountain water for livestock drinking water by using water treatment devices, drinking water is disinfected to inactivate pathogens, chlorination is extremely widespread form of disinfection, while UV light and ozone also utilized around world plant ^[9,10]. Chlorine is closely utilized as disinfectant of drinking water due to it's efficacy, likewise so it reacts with naturalistic organic materials of water leading to create deleterious noxious and complex compounds which know as disinfection by- products (DBPs), in addition the practicable up shot of (DBPs) on health, productive, and reproductive outcomes in animals^[11,12], as an examples of those compounds Trihalomethans, Trichloromethans (chloroform), Bromodichloromethan, Bromochloromethans (Bromoform), Haloacetic acid and more than 700 types^[13,14,15] which found it's high toxic health, Physiological effects and many cancers^[11,13,15,16], also the Trihalomethans and Haloacetic acid, those found in most common form of disinfection by-products in water , else whose concentrations were measured in some countries of the world periodically in water appliances for citizens , also it's used as useful evidence to find out the levels of disinfection by- products in treated water by chlorine and their noticeable adverse effects to function of body systems^[14]. Accordingly , this experiment was conducted to evaluation the effect of chlorine and it's by- products in some hygienic and Physiological traits of male rabbits.

MATERIALS AND METHODS

1- Experiment location:

The experiment was accomplished in animals research farm- Veterinary Medicine College – Baghdad University lasted from 20th November 2017 up to 20th January 2018 to estimate the effect of chlorinated water and its Disinfection by – products(DBPs) in some hygienic and Physiological traits of male rabbits.

2- Experimental design and animals nutrition:

Males rabbits at number and age of twenty- eight, 3 month were checked to verify their health and preventive remedy versus external and internal parasites (Ectopor, Ivermectine, Albendazol, Coccidastop) after create a place for accommodation the animals left 3 weeks for adaptation, body weight was considered in division, also all groups animals fed concentrated diet pellets 75gm/head according to feed requirements of American research council^[17], alfalfa and treated water offered freely, also animals arranged into four groups(7 each) as followings first group drinking chlorine free water as a negative control, second drinking tap water (chlorine estimated at rate 0.05 ppm/L) as a positive control, third(chlorine 3ppm/L water) (distilled water treated with chlorine), fourth(chlorine 5 ppm/L water).

3- Blood samples, traits and physiological assay:

Blood samples were taken biweekly by direct from the heart, serum obtained for blood traits and Physiological analysis that which included hemoglobin(Hb), packed cell volume(PCV) total protein by using spectrophotometric method and Haematocrite value according^[18,19,20,21], malondialdehyde(MDA), glutathione by using (ELISA) according^[22], also by using Reflotron apparatus to measurement transaminase enzymes (AST,ALT), alkaline phosphate and bilirubin levels by commercially available kits^[20,21,24].

4- Chemical implementation:

Chlorinated water at concentration* (3ppm/L and 5 ppm/L) prepared previously before each use for animals groups were treated with chlorinated water^[23]. Tap water quality criteria investigate to assessment the liberated chlorine levels as(ppm/L) by gathering samples of water and using pocket digital colorimeter that analysis water samples for liberated chlorine by following the instruction of using, likewise the device is modern technology with high

* Aqua tabs: 1 tab/20L of water(Ireland made) Ministry of Health / Iraq

sensitivity about range from 0.01 up to 1.00 ppm/L^[25], also the disinfection by- products (DBPs) (Trihalomethanes) in tap and treated water measured by using (HPLC) high performance liquid chromatography technique according^[26,27].

5- Statistical analysis:-

The outcomes data were analyzed as a Complete Randomized Design(4 treatment) (CRD) , Least significant differences (LSD) was utilized to reveal the significant differences amongst different groups means at ($P < 0.05$) level^[28].

RESULTS AND DISCUSSION

Effect of different processing in some blood traits:

1) Blood hemoglobin (Hb)g/dl

The outcomes be visible significant ($p < 0.05$) differences among the treated groups at different periods. The tap water group(2nd) exhibited significantly ($p < 0.05$) higher estimates (17.33 ± 0.04) than other groups in blood hemoglobin(table1), while the chlorine free water group (1st) share out the second group in recording higher significant differences (14.43 ± 0.41), at same time the chlorine 3ppm, 5ppm water groups (3rd, 4th) showed a gradual decreasing viz significantly lower during experiment pheriods (11.80 ± 0.49) (11.04 ± 0.81) respectively and in particular at the end of experiment.

2) Paked Cell Volume (PCV%)

The same tendency exhibited in PCV values as in the Hb (table2) , the second group exhibited significant ($p < 0.05$) higher estimates (42.89 ± 0.69) than other groups, while the first group share out the second group in recording higher differences (39.03 ± 0.75), while the third and fourth group exhibited a gradual decreasing viz significantly lower throughout experiment periods (30.04 ± 0.55)(29.54 ± 0.75) respectively and in particular at the end of experiment.

Table 1: Effect of treated water in hemoglobin concentration (Hb)(g/dl) of male rabbits (means \pm SE)

Period Biweekly Treated groups	Adaptation Period	1 st period	2 nd period	3 rd period	4 th period
Chlorine free water 1 st group	B 13.72 \pm 0.73	A 14.95 \pm 0.51	B 15.07 \pm 0.91	B 14.11 \pm 0.88	B 14.43 \pm 0.41
Tap water 2 nd group	A 14.01 \pm 0.12	A 15.22 \pm 0.29	A 16.41 \pm 0.87	A 16.32 \pm 0.03	A 17.33 \pm 0.04
Chlorine 3ppm water 3 rd group	A 14.90 \pm 0.55	B 13.75 \pm 0.03	BC 12.83 \pm 0.46	C 12.02 \pm 0.57	C 11.80 \pm 0.49
Chlorine 5ppm water 4 th group	B 13.95 \pm 0.37	C 13.05 \pm 0.88	C 12.01 \pm 0.35	C 11.71 \pm 0.49	C 11.04 \pm 0.81

Different capital letters vertically indicate significantly different results ($p < 0.05$)

Table 2: Effect of treated water in blood packed cell volume (pcv%) of male rabbits (means \pm SE)

Period Biweekly Treated groups	Adaptation Period	1 st period	2 nd period	3 rd period	4 th period
Chlorine free water 1 st group	A 35.41 \pm 0.47	A 35.89 \pm 0.61	AB 36.74 \pm 0.83	AB 37.72 \pm 1.01	B 39.03 \pm 0.75
Tap water 2 nd group	A 35.91 \pm 1.00	A 37.13 \pm 0.81	A 38.97 \pm 0.93	A 41.49 \pm 0.88	A 42.89 \pm 0.69
Chlorine 3ppm water 3 rd group	A 35.78 \pm 0.13	B 34.92 \pm 0.79	B 33.39 \pm 0.25	B 31.98 \pm 0.62	C 30.04 \pm 0.55
Chlorine 5ppm water 4 th group	A 35.83 \pm 1.09	B 34.01 \pm 0.89	B 32.47 \pm 0.56	C 30.17 \pm 0.58	C 29.54 \pm 0.76

Different capital letters vertically indicate significantly different results ($p < 0.05$)

The gradually increased in estimates of blood traits Hb and PCV table (1,2) in the treated groups particularly second group could be attributed to the concentration of chlorine in water of this group within the allowable percentages at treated water lead up to provide a hygienic environment in the digestive tract of animals, also this will lead to rising the absorption of nutrients in the intestine, as another it's resulted more metabolic activation in animal and stimulate erythrocytes synthesis due to synthesis of the erythropoietin in haemopoietic tissue of bone marrow, this outcomes agreed with result obtained by some researches^[1,29,30,31,32]. On the other hand, the decline in those components mostly in the third, fourth groups could be attributed to the high portions of chlorine and it's disinfection by- products (DBPs) due to disinfection the water by chlorine and overlapping or suppression the excretion of erythropoietin hormone from the kidney and with a low portions from the liver, which the first and most responsible for synthesis of erythrocytes from bone marrow, and therefore lowering in Hb, PCV estimates, due to the most organs are exposed to the effect of disinfection by- products (DBPs) are kidney and liver, likewise may be at the brain level and it's toxicity effects in addition to the stress caused by (DBPs) on all body, but perhaps the effects exceed to level of cell, or may be exceed in toxicity and effect on cell genome, this a greed and confirmed with ^[33,34,35], also the reduction in blood component may be attributed to (DBPs) and it's effect in some enzymes related with RBC synthesis and therefore effect in age of RBC and increase it fragility exposing RBC to hemolysis, therefore causing anemia, also the interaction of (DBPs) with the action of enzymes result from oxidative stress which caused by (DBPs) and the RBC more exposed free radicals (ROS) causing oxidative destruction to walls of RBS through a great series of hemolytic disorders in RBC, this confirmed and agreed with ^[33] especially in third and fourth groups (3ppm, 5ppm) respectively ^[33,35]

Effect of different processing in some oxidative stress traits:

Malondialdehyde (MDA) concentration (nmol/ml³):

The outcomes mentioned significant ($p < 0.05$) differences among the treated groups at different periods. The third (chlorine 3ppm), fourth (5ppm) groups exhibited significantly ($p < 0.05$) higher estimates (1.06 ± 0.01) (1.09 ± 0.03) respectively than first and second groups in (MDA) concentration (table 3), while the first, second groups exhibited significantly lower than third and fourth groups espically at the end of experiment.

Glutathione Concentration ng/ml³:

Table (4) mentioned the third and fourth groups exhibited significantly ($p < 0.05$) higher estimates in glutathione concentration than first and second groups (0.27 ± 0.02) (0.30 ± 0.03) respectively especially at adaptation periods, but at the end of experiment the third and fourth groups recorded significantly lower than first and second groups, at the same time the first and especially second groups recorded significantly ($p < 0.05$) higher estimates (0.31 ± 0.05) (0.29 ± 0.06) respectively than other groups.

Table 3: Effect of treated water in Malondildehyde (MDA) concentration nmol/ml³ of Male Rabbits (means \pm SE)

Period Biweekly Treated groups	Adaptation Period	1 st period	2 nd period	3 rd period	4 th period
Chlorine free water 1 st group	A 0.61 ± 0.03	A 0.82 ± 0.01	B 0.78 ± 0.02	B 0.82 ± 0.03	B 0.85 ± 0.01
Tap water 2 nd group	C 0.55 ± 0.02	B 0.79 ± 0.01	C 0.76 ± 0.02	C 0.78 ± 0.01	C 0.81 ± 0.02
Chlorine 3ppm water 3 rd group	B 0.59 ± 0.01	B 0.75 ± 0.02	A 0.91 ± 0.01	A 0.96 ± 0.02	A 1.06 ± 0.01
Chlorine 5ppm water 4 th group	B 0.59 ± 0.03	A 0.83 ± 0.02	A 0.92 ± 0.01	A 1.05 ± 0.02	A 1.09 ± 0.03

Different capital vertically indicate significantly different result ($p < 0.05$)

Table 4: Effect of treated water in Glutathione concentration ng/ml³ of Male Rabbits (means \pm SE)

Treated groups \ Period Biweekly	Adaptation Period	1 st period	2 nd period	3 rd period	4 th period
Chlorine free water 1 st group	D 0.23 \pm 0.02	B 0.25 \pm 0.03	B 0.27 \pm 0.02	B 0.28 \pm 0.01	B 0.29 \pm 0.06
Tap water 2 nd group	C 0.25 \pm 0.01	A 0.27 \pm 0.03	A 0.28 \pm 0.07	A 0.29 \pm 0.05	A 0.31 \pm 0.05
Chlorine 3ppm water 3 rd group	B 0.27 \pm 0.02	C 0.24 \pm 0.07	D 0.23 \pm 0.04	C 0.20 \pm 0.05	C 0.19 \pm 0.03
Chlorine 5ppm water 4 th group	A 0.30 \pm 0.03	A 0.27 \pm 0.04	C 0.24 \pm 0.03	D 0.21 \pm 0.01	D 0.18 \pm 0.02

Different capital letters vertically indicate significantly different results ($p < 0.05$)

The outcomes of this study (Table 3) revealed significantly ($p < 0.05$) higher in malondialdehyde concentration especially in male rabbit serum that treated water at contrition (3ppm, 5ppm) (3rd, 4th groups) compared with another groups could be attributed to the portability of (DBPs) to activating the oxidation and producing the free radicals and increasing fat peroxidation leading to oxidative stress, therefore increasing malondialdehyde concentration as a important production of peroxidation, this outcomes agreed with [33, 36], also several studies have indicated to direct relation between oxidative stress while exposed and taking DBPs for long – term and it's toxic effect [12, 37, 38], likewise the higher outcomes of MDA could be attributed to the DBPs and it's effect, activating to enzymes that in change of unsaturated fatty acid oxidation, which lead to producing hydrogen peroxide and therefore disorder in balance between production of free radicals and antioxidant defenses which caused tissues damage and increased MDA production, while any higher record in MDA level in groups that treated with free chlorine water and tap water on this animals didn't exposed to any oxidative stress during experiment periods [30, 31, 39] As for glutation (table 4) and when the first, second groups recorded significantly ($p < 0.05$) higher estimates than other groups especially at end of experiment it could be attributed to animals of those groups under this treatment and approved chlorine

concentration may enjoyed good health and wasn't exposed to any oxidative stress which causes decline in glutation level, while the reduction in glutation epically in 3rd, 4th groups could be attributed to the excess of chlorine and (DBPs) level in water, those are considered more toxic, gangerous pollutants and stressers, likewise as a result of stress and producing of ROS and free radicals (Reactive Oxygen Spicies) which those depleted the glutation as a antioxidant, or may be this DBPs had high affinity with active groups of enzymes and defeating it's effectiveness [11,33,40,41,42,43]

Effect of different processing in some Physiological traits and enzymes activity (liver function):

Total protein concentration gm /dl :

The outcomes mentioned (table 5), the first and second groups had significantly ($p < 0.05$) higher values than other groups espically at the begining of experiment, also it's adjective from the outcomes of second groups that the total protein concentration seems to increased and recording significantly ($p < 0.05$) higher than all groups at the end of experiment (6.82 ± 0.02), also the trend of total protein concentration seems to decreased in 3rd, 4th groups until end of experiment.

Table 5: Effect of treated water in total protein concentration g/dl of Male Rabbits (means \pm SE)

Period Biweekly Treated groups	Adaptation Period	1 st period	2 nd period	3 rd period	4 th period
Chlorine free water 1 st group	A 5.71 \pm 0.01	A 5.90 \pm 0.02	A 5.83 \pm 0.01	AB 5.89 \pm 0.01	B 5.03 \pm 0.01
Tap water 2 nd group	AB 5.49 \pm 0.03	A 5.79 \pm 0.01	A 5.99 \pm 0.02	A 6.09 \pm 0.03	A 6.82 \pm 0.02
Chlorine 3ppm water 3 rd group	B 5.01 \pm 0.02	AB 5.03 \pm 0.01	B 4.91 \pm 0.02	B 4.70 \pm 0.02	B 4.12 \pm 0.03
Chlorine 5ppm water 4 th group	A 5.85 \pm 0.02	AB 5.07 \pm 0.01	AB 5.01 \pm 0.02	C 4.31 \pm 0.02	C 3.91 \pm 0.01

Different capital letters vertically indicate significantly different results ($p < 0.05$)

The gradually decreased in total protein concentration in 3rd, 4th groups treated with water contain high concentration of chlorine and DBPs (table 5) it may be attributed to the toxic effect of this by- products on protein manufacturer hepatic cell, therefore impact the total protein concentration, viz decrease in protein synthesis and increase catabolism of protein, also through the oxidative stress and hepatic injury induced by this by- products, similar results were agreed and confirmed by some researches ^[44,45], while the progressively increased in outcomes of total protein espically in 1st, 2nd groups wheatear at adaptation up to end of experiment could be mentioned to the role of chorine as a disinfectant in approved percentage at tap water could be promote body health systems and body organs like liver which is considered one of the vital organs in the body and it's role in protein synthesis components ^[46, 47], likewise the decrease in total protein concentration could be attributed to attachment of the DBPs with some minerals that connection with some proteins that responsible about detoxification in liver and this may be impact enzymes in change of synthesis of proteins in liver ^[44, 48].

[2] Aspartate transaminase (AST) IU/L [3] Alanine transaminase (ALT) IU/L

[4] Alkaline phosphate (ALP) enzyme IU/L [5] Bilirubin concentration (mm/dl)

The third (chlorine 3ppm) and fourth (chlorine 5ppm) groups exhibited significantly ($p < 0.05$) higher outcomes than the first(chlorine free water), second(Tap water) groups from adaptation period up to end of experiment table (6-9) in liver activity enzymes(AST, ALT, ALP, Bilirubin) and the same time similar trends were obtained in those enzymes negatively in first and second groups up to end of experiment, also some stability of values at some times of this enzymes and those values were $(53.92 \pm 0.65, 59.12 \pm 1.60)(79.17 \pm 1.13, 84.85 \pm 1.21) (73.15 \pm 0.43, 78.90 \pm 1.02)$ respectively.

Table 6: Effect of treated water in Aspartate transaminase enzyme (AST) IU/L of local male Rabbits(means \pm SE)

Period Biweekly Treated group	Adaptation period	1 st period	2 nd period	3 rd period	4 th period
Chlorine free water 1 st group	B 33.51 \pm 0.81	B 35.75 \pm 0.69	C 32.44 \pm 0.62	C 30.96 \pm 0.76	C 29.18 \pm 0.43
Tap water 2 nd group	A 35.76 \pm 0.34	C 33.14 \pm 1.01	D 30.59 \pm 0.98	D 28.31 \pm 0.07	D 26.13 \pm 0.86
Chlorine 3ppm water 3 rd group	AB 34.17 \pm 0.09	A 37.94 \pm 0.91	B 41.17 \pm 0.83	B 47.09 \pm 0.76	B 53.92 \pm 0.65
Chlorine 5ppm water 4 th group	AB 34.85 \pm 0.21	A 38.09 \pm 0.57	A 43.92 \pm 0.17	A 49.81 \pm 1.09	A 59.12 \pm 1.60

Different capital letters vertically indicate significantly different results ($p < 0.05$)

Table 7: Effect of treated water in Alanine transaminase enzyme (ALT) IU/L of local male Rabbits(means \pm SE)

Period Biweekly Treated group	Adaptation period	1 st period	2 nd period	3 rd period	4 th period
Chlorine free water 1 st group	C 54.17 \pm 0.91	C 56.31 \pm 1.69	C 58.11 \pm 1.02	C 60.41 \pm 0.87	C 61.33 \pm 1.08
Tap water 2 nd group	B 55.81 \pm 1.02	D 48.61 \pm 1.71	D 41.87 \pm 1.37	D 40.91 \pm 0.97	D 40.01 \pm 0.83
Chlorine 3ppm water 3 rd group	A 57.01 \pm 1.09	A 63.13 \pm 1.03	B 67.51 \pm 1.21	B 72.41 \pm 0.98	B 79.17 \pm 1.13
Chlorine 5ppm water 4 th group	A 56.09 \pm 1.13	A 61.76 \pm 1.51	A 69.83 \pm 1.43	A 76.78 \pm 1.37	A 84.85 \pm 1.21

Different capital letters vertically indicate significantly different results ($p < 0.05$)

Table 8: Effect of treated water in Alkaline phosphate enzyme (ALP) IU/L of Male Rabbits(means \pm SE)

Period Biweekly Treated group	Adaptation period	1 st period	2 nd period	3 rd period	4 th period
Chlorine free water 1 st group	B 48.07 \pm 0.31	BC 47.99 \pm 0.85	C 44.81 \pm 0.77	C 14.97 \pm 0.57	C 39.09 \pm 0.76
Tap water 2 nd group	A 49.12 \pm 0.70	B 45.27 \pm 0.84	D 40.94 \pm 1.02	D 38.94 \pm 0.92	D 35.33 \pm 0.79
Chlorine 3ppm water 3 rd group	A 50.01 \pm 0.21	A 59.91 \pm 1.00	B 64.87 \pm 0.65	B 68.95 \pm 0.74	B 73.15 \pm 0.43
Chlorine 5ppm water 4 th group	A 50.11 \pm 0.48	B 55.01 \pm 0.95	A 66.17 \pm 0.57	A 74.71 \pm 0.81	A 78.90 \pm 1.02

Different capital letters vertically indicate significantly different results ($p < 0.05$)

Table 9: Effect of treated water in Billrubin concentration ((mmol/dl) of local Male Rabbits(means \pm SE)

Period Biweekly Treated group	Adaptation period	1 st period	2 nd period	3 rd period	4 th period
Chlorin free water 1 st group	A 58.01 \pm 0.02	B 60.11 \pm 0.03	C 61.43 \pm 0.02	C 62.47 \pm 0.03	C 61.35 \pm 0.01
Tap water 2 nd group	B 55.17 \pm 0.03	C 56.31 \pm 0.02	D 57.16 \pm 0.01	D 55.84 \pm 0.03	D 56.19 \pm 0.03
Chlorine 3ppm water 3 rd group	A 56.02 \pm 0.01	A 63.59 \pm 0.01	B 66.88 \pm 0.02	B 69.14 \pm 0.03	B 70.92 \pm 0.02
Chlorine 5ppm water 4 th group	B 55.93 \pm 0.06	A 64.93 \pm 0.03	A 69.90 \pm 0.02	A 73.19 \pm 0.02	A 76.79 \pm 0.02

Different capital letters vertically indicate significantly different results ($p < 0.05$)

The progressively increased in the outcomes of AST, ALT, ALP, Bilirubin in treated groups with chlorine (3ppm, 5ppm) and high percentages of DBPs especially in third, fourth groups table (6-9) compared with first, second groups during experiment duration could be attributed to the oxidative stress in liver and the injury done to tissues that contains those enzymes especially liver cells resulting in production free radicals (ROS) that be impact in cell activity especially cell wall functions, as considered DBPs as toxic and harmful impact materials to cell functions, also this DBPs had ability and high affinity with cell wall when exposed to DBPs resulting in induced many changes in function and composition of cell wall through increasing permeability and therefore leakage of enzymes to blood stream, or may be through intervention of those by-products DBPs and chlorine with enzymatic systems and synthesis process for those enzymes, or by depletion of antioxidants, these results confirmed and work in with previous results obtained by some studies [44,45,49,50,51,52,53]

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