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Ingestion of chlorinated water has no effect upon indicators of cardiovascular disease in pigeons

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Summary

Cardiovascular disease (CVD) accounts for nearly half the deaths, yearly, in the United States. The arterio(athero)sclerotic plaque is the principal lesion of CVD. The White Carneau (WC) pigeon is an animal model that has been employed extensively for studying CVD. Cholesterol (CHOL) feeding aggravates atherosclerosis in WC pigeons > 2 years old. In 1986, two reports appeared from a single laboratory claiming a direct effect of drinking chlorinated (Cl) water upon lipid levels and plaque development in young (< 1 year) WC pigeons. These are the only reports of such direct effects, to date. Three months' exposure to 2 ppm or 15 ppm Cl in the drinking water, resulted in increased circulating CHOL levels in young male WC pigeons fed a normocholesterolemic (NC) diet in which Ca^{2+} levels were reduced. In addition, at both Cl concentrations there was a significant increase in plaque size, compared to controls. Pigeons in the 2 ppm group also exhibited elevated low density lipoprotein (LDL) levels after 3 months on the NC diet. These findings, if extrapolated to man, could have considerable public health consequences, since nearly 200 million people in the United States drink Cl water. We have carried out a similar set of studies but with strikingly different results. We used the same suppliers of pigeons and feed as did the authors of the 1986 reports and followed their approach where possible. Six month-old male WC pigeons drank water with 2 ppm or 15 ppm Cl (pH 8.5) and ate a NC diet with Ca^{2+} reduced to 80% of normal. At both 1 and 3 months, body weight, CHOL, triglyceride and LDL levels were unaffected by drinking Cl water. There was also no effect of Cl water on plaque size after 3 months. Thus, we found no evidence that drinking chlorinated water has any effect upon circulating lipid levels or upon the development of arteriosclerotic plaques, in this animal model.

Key words: Pigeons; Chlorinated water; Dietary Ca^{2+}; Lipids; Arteriosclerotic plaques

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States, accounting for nearly half of all deaths yearly [1]. The principal lesion

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associated with this disease is the arteriosclerotic plaque. Plaques arise partly as a result of smooth muscle cell (SMC) proliferation in the intimal region of the artery wall, which lies between the single layer of lumenal facing endothelium and the medial (SMC) layer.

There is strong epidemiological evidence for an interaction of genetic and environmental factors (e.g. cigarette smoke, diesel exhaust, etc.) in the development of clinically significant CVD. During the past decade, research on two avian models has demonstrated that environmental mutagens and carcinogens may play roles in both the onset and development of arteriosclerotic plaques (see specific references below). Both cockerels and pigeons have proven to be especially effective animal models for studying arteriosclerotic plaque development. In cockerels, spontaneous plaques which develop in the abdominal aorta are very similar morphologically and ultrastructurally to fibromuscular coronary artery plaques in humans [2]. Development of cockerel plaques is augmented by the presence of environmental agents. Accelerated plaque development results from weekly administration of polycyclic aromatic carcinogens at sub-tumorigenic doses [3—7]. In addition, a single injection of the oncogenic herpes virus, Marek disease virus, into otherwise untreated cockerels results in the appearance of plaques in the thoracic aorta [8]. These herpes-associated plaques contain elevated levels of cholesterol and cholesterol esters [9].

Among pigeons, the White Carneau (WC) breed is most often used in atherosclerosis studies because it responds to cholesterol feeding by exhibiting aggravated atherosclerosis [10,11]. In older birds (>2 years of age), this condition closely resembles many of the aspects of the human disease [12].

For most of this century, chlorine has been widely used as a disinfectant to control water borne infectious disease in public drinking water in the United States. More than 190 million Americans drink chlorinated water. According to Environmental Protection Agency data, as of 1975, the majority of these people obtained chlorinated water from community water systems [13]. Given the vast numbers of people exposed to this disinfectant, it is encouraging that no major public health risks associated with water chlorination have been identified. In fact, until recently there were only two isolated reports which suggested possible indirect health risks associated with water chlorination. Potentially harmful chlorinated compounds, such as trihalomethane (reviewed in Ref. 14), produced by interaction of chlorine with organic matter, are sometime present in drinking water treated with chlorine, albeit at low concentrations. Also, one set of epidemiologic data suggested that a link may exist between ingestion of chlorinated water and levels of CVD in man [15]. On the other hand, a 1982 study showed no effects of chlorine or any of four other water chlorinating agents on serum cholesterol levels in humans [16]. A 1986 epidemiological study failed to find any association between deaths from CVD and exposure to chlorinated drinking water. Mortality records from >200,000 people were used for this study [17]. However, also in 1986, two reports appeared from one laboratory claiming a direct effect of drinking chlorinated water upon lipid levels and plaque development in experimental animals [18,19]. These were the first and so far, only reports of such direct effects. In one report, 3 months of exposure, beginning at

4 months of age, to 2 ppm or 15 ppm chlorine, or monochloramine or chlorine dioxide, each at 2 ppm, resulted in increased circulating cholesterol levels in male WC pigeons [18]. In addition, chlorine at 2 ppm and 15 ppm caused significant increases in plaque size in male WC pigeons on a normal lipid diet, compared to controls. Low density lipoprotein (LDL) levels were significantly increased in pigeons fed normal or hyper-cholesterolemic diets for 3 months while being exposed to 2 ppm chlorine in the drinking water. The effects of chlorine on lipid levels and plaque size were observed only when the dietary calcium level was reduced to 0.35%, which was described as 80% of the minimum daily requirement (MDR) for pigeons [18].

In the second report presented at the 1985 Symposium on the Health Effects of Drinking Water Disinfectants [19], statistically significant increases in cardiac hypertrophy and in hydroxyproline levels were found in male WC pigeons that drank water with 10—30 ppm chlorine for 9 months beginning at 4 months of age. The increases in hydroxyproline levels were interpreted as representing an increase in cardiac connective tissue.

Although these are the only reports claiming a direct effect of chlorinated water ingestion upon the development of CVD, the potential public health implications warranted an independent attempt to confirm these findings. We report here that we have been unable to confirm any of these findings.

Materials and methods

Animals

Animals and feed were purchased from the same vendors used in the original reports [18,19]. Male WC pigeons (6—7 months of age) were purchased from the Palmetto Pigeon Plant, Sumter, SC. Following a 2-week quarantine period, pigeons were assigned randomly into 3 groups (control, 2 ppm chlorine, 15 ppm chlorine). Pigeons were housed in stainless steel pigeon cages (3 per cage) that meet AALAC specifications. Initially, there were 17 pigeons assigned to each treatment group and 16 assigned to the control group. At various times after the experiments began, 4 birds from each group were selected at random for ultrastructural studies (to be described elsewhere). Thus, at the end of the experiments there were 13 cockerels left in each treatment group and 12 in the control group. All were fed a standard pigeon chow diet, low in cholesterol and saturated fat (Purina, St. Louis, MO), except that dietary calcium was reduced from 0.90% to 0.72%. Animals were weighed weekly.

Water

In the 1986 studies, statistically significant effects of drinking chlorinated (both 2 ppm and 15 ppm chlorine) water (pH 8.5) upon lipid levels and/or plaque size were reported [18]. We chose, therefore, to test the same two chlorine concentrations here. Chlorinated drinking water was prepared fresh daily by the methods [20,21] referred to by Revis et al. [18]. Sodium hypochlorite was added to distilled, deionized drinking water to the appropriate final concentrations. All drinking water was kept buffered to pH 8.5 with a bicarbonate buffer. Water

was made available to each group of pigeons via a manifold system constructed from PVC tubing, connected at one end to a Nalgene reservoir and at the other end, via a stainless steel valve, to PVC cups (Edstrom, Waterford, WI). The maximum volume available from each cup was 5 ml. To minimize evaporation the reservoirs were kept covered and the cups were constructed so that the stainless steel valve had to be depressed (either manually or by the birds) before water flowed from the reservoir to the cup. The cups were filled with freshly prepared water each morning. Cups were refilled from the reservoirs 6 h later. Six hours after preparation, chlorine concentrations in the reservoirs were 90—100% of their values at the time of preparation. Daily water consumption was recorded for each group. Pigeons in the 2 ppm and 15 ppm groups drank chlorinated water only, throughout the study. Food and water were available to all groups ad libitum. However, animals were starved overnight before blood samples were taken.

Blood collection and lipoprotein isolation

Blood was collected from the alar veins into non-heparinized syringes. Serum lipoproteins were isolated by sequential flotation in salt gradients prepared with sodium chloride and sodium bromide [22]. All samples with a density < 1.007 g/ml were classed as VLDL, those between 1.007 and 1.063 g/ml as LDL, and all those with a density > 1.063 g/ml as HDL. Following delipidation [23], the identity of each lipoprotein fraction, isolated by ultracentrifugation, was confirmed by SDS gel electrophoresis [24].

Lipid determinations

Cholesterol levels were determined by an enzymatic (cholesterol oxidase) method on whole serum as well as on individual VLDL, LDL, and HDL samples from each animal [25]. Triglycerides in the serum and in each of the isolated lipoprotein fractions were also determined colorimetrically [26]. Cholesterol and triglyceride levels were measured at 0, 1, and 3 months. LDL/HDL levels were measured at 1 and 3 months.

Plaque morphometry

Four pigeons from each group were set aside for ultrastructural studies. Plaque morphometry was carried out on 13 pigeons from each test group and 12 from the control group. Immediately after sacrifice, each aorta was removed, washed in warm buffered saline, cleaned of excess connective tissue and fixed in phosphate buffered formalin (pH 7.4). Each aorta was coded before sectioning and the code was not broken until after all relative plaque thicknesses were determined. The entire aorta from each pigeon was sectioned transversely into 3-mm segments from the iliac arteries to the aortic arch. Following paraffin embedding, 5 μM thick sections were cut from each 3-mm segment and stained by the Verhoeff-van Gieson procedure [27] which identifies elastin and collagen and allows the medial (SMC) layer and the outer adventitial (connective tissue) layer of the artery wall to be distinguished readily from each other and from plaque, where it exists.

A point counting method was used [28] to determine plaque cross-sectional area (x.s.) relative to that of the medial layer of the artery wall. A magnified (250×) image of the arterial section was projected onto a viewing screen attached to a Zeiss microscope. A grid of equally spaced points was superimposed upon the magnified image. The number of points counted was a function of the x.s. area of the section. By including measurements of intimal and medial cross-sectional areas for each segment, artifacts due to staining and sectioning were minimized. For each aorta, the relative plaque size was calculated as the sum of relative plaque areas of each of the 3-mm segments ((intimal (plaque) cross-sectional area divided by the intimal + medial cross-sectional area) × 100).

Statistical analysis

Lipid levels were compared by a one factor analysis of variance (ANOVA) for all three groups at each sampling period and within each group for all sampling periods. Significant differences (at 95%) were determined by Dunnett's *t*-test. In addition, lipid levels in those animals sampled at all three time points were compared by the repeated measures ANOVA. When significant differences between groups were detected, these values were further compared by the Tukey Multiple Comparison Procedure [29]. Body weights were compared by the non-parametric Kruskal-Wallis test.

Results

For three months, male WC pigeons (approximately 8 months old at 0 time) were fed a standard mash diet with calcium levels reduced to 80% of normal and were given drinking water containing chlorine at 0 ppm, 2 ppm or 15 ppm. The effects of this treatment upon body weights, serum cholesterol and triglyceride levels, LDL/HDL ratios, and arteriosclerotic plaque size were determined. There were no significant differences detected in any of these parameters, either between or within groups, that could be attributed to the ingestion of chlorinated water.

Animal weights

Animals were weighed weekly. There were no differences in weights between any of the groups at any of the sampling times (data not presented).

Water consumption

Each day water levels in the reservoirs were measured immediately before the reservoirs were refilled with freshly prepared water. The amount consumed thus includes water that was drunk plus small amounts that were spilled or that evaporated. For each group the total volume consumed divided by the number of birds provides a rough estimate of the amount of water each bird consumed. Each bird (control and experimental) consumed 70—75 ml of water per day (data not presented).

It is difficult to determine directly how much chlorine each bird actually received. One hour after cups were filled with freshly prepared chlorinated water,

the chlorine concentrations in the cups were 70—80% of the initial values. After 6 h the chlorine concentrations in the small amount of water (approx. 1 ml) remaining in the cups were 40—60% of the starting values. The cups were refilled after 6 h with full concentration chlorine solutions. Thus, at the very least (assuming instantaneous loss of 20—30% of the chlorine from the cups and further assuming that the cups were never refilled) the pigeons were exposed to 70—80% of the prepared dose during the first hour and approximately 50% of the initial dose 6 h later. Since even 6 h after preparation, the reservoir water had chlorine concentrations 90—100% of the starting values, any reservoir water added to the cups at this time was equivalent to freshly prepared water, in terms of chlorine concentration.

Lipid levels

Serum cholesterol

At 0 time, the pigeons were randomly distributed into three groups (17 pigeons in each test group and 16 in the control group). Before any exposures were begun, serum cholesterol and triglyceride levels were measured in 28 of the 50 pigeons to provide baseline values. Of these 28 pigeons, 11 were from the group that became controls, 10 were from the group that became the 2 ppm group, and 7 were from the group that became the 15 ppm group. Cholesterol levels were determined in all pigeons from each group at 1 and 3 months. The serum cholesterol values for each group at 0, 1, and 3 months, for pigeons sampled all three times, are presented in Table I. Comparison of 0 time values among the three groups was made by a one factor ANOVA. Prior to carrying this out, the data were tested for normality of distribution, homogeneity of variance, and normality of residuals. The one factor ANOVA on the 0 time cholesterol readings indicated that a significant difference existed among groups ($P = 0.049$). The more rigorous Tukey Multiple Comparison Procedure confirmed this and showed that for

TABLE I

SERUM CHOLESTEROL VALUES OF PIGEONS SAMPLED AT ALL THREE TIME POINTS (mg/dl; mean ± S.E.M.)

	0 time	1 month	3 months
Control	279.0 ± 16.9 ($n = 11$)	278.3 ± 15.3 ($n = 11$)	285.5 ± 21.4 ($n = 11$)
2 ppm	249.6 ± 10.3[a] ($n = 9$)	267.4 ± 12.7 ($n = 9$)	274.7 ± 13.6 ($n = 9$)
15 ppm	311.4 ± 19.4[a] ($n = 7$)	329.2 ± 23.7 ($n = 7$)	346.4 ± 9.8 ($n = 7$)

[a]Significant differences at 0 time between 2 ppm and 15 ppm ($P < 0.05$), repeated measures ANOVA; Tukey's Multiple Comparison Test. Subsequent chlorine exposures resulted in no significant changes in serum cholesterol levels. There were no significant differences ($P > 0.05$) within groups.

birds measured all three times, the 0 time cholesterol values in the 15 ppm group were significantly higher than those in birds from the 2 ppm group.

Despite these differences between the 2 ppm and 15 ppm groups at 0 time, the subsequent chlorine treatments were not responsible for eliciting any significant change in serum cholesterol levels in either group. In the 15 ppm group, where the largest gap existed between the means of the 0 time values (311.4 ± 51.4 mg/dl) and the 3 month values (346.4 ± 26.2 mg/dl) for pigeons measured all three times, these differences were not significant ($P > 0.05$). There were also no significant differences between the 0 time and 3 month mean values for either the 2 ppm group or the controls ($P > 0.05$). Within any of these groups there were also no significant differences in serum cholesterol levels between 0 time and 1 month or between 1 month and 3 months.

When comparisons between groups were made at 0, 1 and 3 months, the difference between the 2 ppm and 15 ppm group at both 0 time and 1 month was 61.8 mg/dl and after 3 months, the difference was 71.7 mg/dl. These three sets of values are not significantly different from each other ($P > 0.05$).

These data indicate that neither continuous short-term exposure (0 time to 1 month) nor continuous longer exposure (0 time to 3 months) to chlorinated drinking water caused significant increases in serum cholesterol levels.

Limiting the analysis to only those animals sampled at all three time points (Table I) is the most rigorous approach statistically, even though it excludes a number of animals from the analysis. If instead, all the animals in each group at 1 and 3 months are compared to their respective 0 time controls, the results are unchanged. The small increases in serum cholesterol levels cannot be attributed to exposure to chlorinated drinking water (data not presented).

Triglyceride levels

Serum triglyceride levels in the three groups at each time point are presented in Table II. At none of the three time points was there a statistically significant difference (at 95%) between control and experimental triglyceride values. (Results of

TABLE II

SERUM TRIGLYCERIDE VALUES OF PIGEONS SAMPLED AT ANY TIME POINTS (mg/dl; mean ± S.E.M.)

	0 time	1 month	3 months
Control	205.1 ± 13.1 (n = 11)	162.1 ± 10.1 (n = 10)	195.1 ± 11.1 (n = 14)
2 ppm	212.4 ± 22.1 (n = 10)	195.7 ± 19.4 (n = 15)	195.4 ± 19.7 (n = 10)
15 ppm	196.7 ± 27.4 (n = 8)	181.7 ± 12.7 (n = 14)	199.3 ± 12.0 (n = 14)

There were no significant differences detected ($P > 0.05$), one-factor ANOVA.

TABLE III

LDL/HDL CHOLESTEROL VALUES OF PIGEONS SAMPLED AT 1 AND 3 MONTHS (mean ± S.E.M.)

	1 month	3 months
Control	0.256 ± 0.042 (n = 15)	0.280 ± 0.045 (n = 13)
2 ppm	0.229 ± 0.016 (n = 16)	0.200 ± 0.020 (n = 16)
15 ppm	0.261 ± 0.025 (n = 14)	0.253 ± 0.028 (n = 17)

No significant differences were detected ($P > 0.05$), one-factor ANOVA.

a 1 factor ANOVA subjected to Dunnett's t-test). Within control, 2 ppm and 15 ppm groups there were no statistically significant differences in triglyceride levels between any time points

LDL/HDL levels

LDL/HDL values at 1 month and 3 months are presented in Table III. No significant differences existed between groups or within a group ($P > 0.05$).

Aortic arteriosclerosis

Plaque morphometry

In pigeons, aortic plaques develop most prominently in the region of the coeliac bifurcation [30]. In 24 of the 33 pigeons with plaques in this study, plaques appeared at or within 3 mm of the coeliac bifurcation. Plaque size was expressed as the ratio:

$$\frac{\text{plaque x.s. area}}{\text{plaque x.s. area} + \text{media x.s. area}} \times 100$$

There were two striking sets of findings (Fig. 1). First, in no group were values of plaque size strikingly large. In only 13 of the pigeons in the study did relative plaque x.s. area exceed 1% of the x.s. area of the artery wall. Fewer than half of these plaques were visible to the naked eye. Second, there were no significant differences ($P > 0.05$) in relative plaque size between any of the groups. In the control group the values were 0.92% ± 0.37. In the 2 ppm group the values were 1.76% ± 1.14 and in the 15 ppm group the values were 0.76% ± 0.15. The relatively larger values for both mean and variance of plaque size in the 2 ppm group is due to the fact that pigeons with four of the five largest values were in

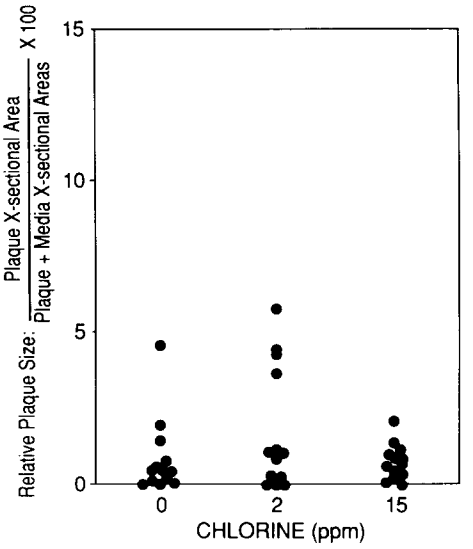

Fig. 1. Effect of chlorinated drinking water on cardiovascular disease in pigeons: scatter plot for plaque data. The percentage of aortic wall x.s. area occupied by plaque was determined for each pigeon (●) in each group after 3-months exposure. An average of 750 points (range 296—1774) from plaque and artery wall were counted for each animal to provide data on relative plaque size. $N = 12$, $N = 13$, $N = 13$ pigeons for 0 time, 2 ppm and 15 ppm, respectively.

this group as were three pigeons that lacked microscopically detectable plaques. In comparison, the control group had one of the five largest plaques and had only one pigeon that lacked microscopically detectable plaques.

Summary

Three-month exposures to chlorinated drinking water (pH 8.5) had no statistically significant effects upon serum lipid levels (cholesterol, triglyceride, LDL/HDL) or plaque size in young male WC pigeons fed a standard pigeon mash diet in which the Ca^{2+} level was reduced to 80% of normal.

Discussion

The objective of these experiments was to determine the effect(s) that compounds used to chlorinate drinking water have upon lipid levels and aortic plaque development, in an experimental animal model, the male WC pigeon. Aortic atherosclerosis in older (> 2 years) WC pigeons bears many similarities to the human disease [30]. In 1986, there were two reports from one laboratory that, under specified test conditions, drinking chlorinated water aggravates the development of CVD in very young male WC pigeons [18,19]. Chlorine (added to the water as sodium hypochlorite) at 2 ppm or 15 ppm was the most effective of the compounds tested. Ingestion of chlorinated water for 3 months was reported to result

in elevated blood cholesterol and triglyceride levels, increased LDL/HDL ratios, and enhanced arteriosclerotic plaque development.

In the studies reported here, the effects of 2 ppm or 15 ppm chlorine in the drinking water upon body weight, levels of circulating cholesterol and triglycerides, and LDL/HDL ratios were determined in young male WC pigeons after 1 and 3 months of exposure. Pigeons were sacrificed at the end of the 3-month exposures and aortic plaque development in pigeons from each test group was compared to that in controls.

Efforts were made to follow the experimental approach of Revis et al. [18,19] wherever possible. In those cases where that could not be done, we tried to keep differences in protocols to a minimum. For example, the youngest male pigeons that we were able to obtain were 6 months old, rather than 3—4 months old as Revis et al. reported using [18]. Additionally, the Ca^{2+} levels in the diets we used were reduced to 80% of the level normally found in pigeon diets (i.e. to approx. 0.70%). The Ca^{2+} level of the feed that Revis et al. used was reported to be 80% of the minimum daily requirement for pigeons (approx. 0.35%).

One other set of differences between our approach and that of Revis et al. bears noting and that is in the area of plaque size determination. Revis et al. [18] state that arteries were stained with the lipid stain Oil Red O, and that surface area of the plaques was measured. Since we tested young pigeons on a low cholesterol, low fat diet, there was no a priori reason to expect that plaques appearing in our test pigeons would be lipid-laden. If the development of largely lipid-free, spontaneous plaques were enhanced by drinking chlorinated water, the size of such plaques would be underestimated by using a lipid stain. Reliable plaque measurement could also be difficult if a lipid stain were used and lipid-laden cells were not uniformly present on the plaque surface. The Verhoeff-van Gieson stain used here permits measurements of plaque size, whether or not lipid is present. Plaques cut in cross section and stained with the Verhoeff stain can be readily distinguished from the medial (SMC) layer of the artery wall so that boundary problems are minimized.

We have found that a reliable way of measuring aortic plaque size is to measure plaque x.s. area relative to that of the underlying artery wall. Arteries are cut into segments of equal length (approx. 3 mm). Each of these segments is cut in x.s. and the cut surface area of the intima and media are compared via a point counting procedure [28]. Artifacts that can arise as a result of fixation and cutting angle are minimized since in any section the intima and media are treated and analyzed together. We have found that this method is more accurate and less prone to error than either measurement of total plaque surface area or of plaque x.s. surface alone. In addition, to minimize observer bias, all aortas were coded prior to sectioning and the code was not broken until all relative plaque areas had been measured.

The main finding of this study is that under the experimental conditions employed, drinking chlorinated water (pH 8.5) has no effect either upon lipid levels or upon arteriosclerotic plaque development in young male WC pigeons. Although there were occasionally significant differences between groups, there was no evidence that these differences were due to the chlorine treatments.

Whether all pigeons in the experiment were evaluated or only those pigeons measured all three times were evaluated, the results were the same. Drinking chlorinated water did not increase serum cholesterol values in either the 2 ppm or 15 ppm groups, compared to controls. Therefore, there were neither age nor treatment effects.

The baseline circulating cholesterol values that we report here are comparable to those cited in the literature [32—34], except for those of Revis et al. [18] which are much lower. Revis et al. also report a wide variability in 0 time cholesterol values. Their 0 time cholesterol value for pigeons that subsequently were fed a low lipid diet was 150 mg/dl while the 0 time cholesterol value for pigeons subsequently fed a high lipid diet exceeded 200 mg/dl [18].

Triglyceride levels in the studies reported here were unaffected by exposure to chlorinated drinking water (Table II). There were no significant differences ($P > 0.05$) within or between treated and control samples. LDL/HDL ratios were measured after 1 month and after 3 months of exposure. There were no significant differences ($P > 0.05$) between or within groups at these time points.

These results demonstrate that under the experimental conditions that we have described here, drinking chlorinated water had no effect upon any of the lipid parameters that were measured. More importantly, there was no significant effect of chlorinated drinking water upon plaque size. We cannot rule out the possibility that longer exposures to chlorine or other chlorinating agents (e.g. monochloramine or chlorine dioxide) could have effects on blood lipid levels or plaque development that were not detectable here after 3 months of exposure to drinking water treated with sodium hypochlorite. Additionally, older pigeons on a high fat diet might be adversely affected by drinking chlorinated water and it would probably be worthwhile to carry out such experiments. Finally, one might argue that exposure to chlorinated drinking water could produce subtle cardiovascular effects that would be discernible if the number of pigeons tested was larger. This argument is often raised when negative results are presented and there is no way to refute this argument directly. However, for each group we tested as many pigeons as were tested per group in the 1986 studies and we found no significant effects of drinking chlorinated water on lipid levels or plaque development. Thus, our results differ from those published by Revis et al. [18].

Conclusion

With this animal model, under the experimental conditions described, drinking chlorinated water (pH 8.5) has no significant effect either upon circulating lipid levels or upon plaque development.

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