individuals, but not by cells of the Bombay type, and saliva from secretors but not from nonsecretors neutralized the precipitating lectin. Furthermore, seed extracts showed a high correlation between hemagglutination titer against red cells of blood group O and the ability to form precipitin bands against saliva of secretors. The results thus leave little doubt that precipitin bands shown between seed extracts and saliva of secretors result from a precipitin reaction between the anti-H lectin and H substance, except for the extract from *Lotus* which was found to contain a lectin other than anti-H in addition to anti-H.

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Chlorinated Urban Water: A Cause of Dialysis-Induced Hemolytic Anemia

Abstract. *Unexplained acute hemolytic anemia is sometimes seen in uremic patients undergoing hemodialysis. Chloramines, which are oxidant compounds made up of chlorine and ammonia and are widely used as bactericidal agents in urban water supplies, have been found responsible for two recent epidemics, in dialyzed uremic patients, of acute hemolytic anemia characterized by Heinz bodies. Chloramines produce denaturation of hemoglobin, both by their direct oxidizing capacity and their ability to inhibit red cell reductive (hexose monophosphate shunt) metabolism.*

Hemolytic anemia is frequently a serious problem among patients undergoing long-term hemodialysis. A few incidents of hemolytic anemia among dialyzed patients have been caused by contaminants such as copper (*1*) and nitrates (*2*) in the dialyzing fluid. We have investigated two renal dialysis facilities where patients manifested obvious hemolytic anemia. The patients' red cells contained large numbers of Heinz bodies, which usually consist of oxidatively denatured hemoglobin (*3*); these inclusions are associated with a decreased survival of red cells (*3, 4*). Reflecting the oxidative denaturation, these patients' red cells also showed increased concentrations of methemoglobin, especially during and immediately after dialysis. Both dialysis units had recently adopted the reverse osmosis (RO) technique of purifying dialysis water. In the reverse osmosis technique a semipermeable membrane is used through which water is sieved at high pressure. This technique removes particulate matter and trace metals such as copper.

The oxidant responsible for this hemolytic anemia was identified primarily through in vitro studies in which small volumes of red cells were exposed to large volumes of test waters made isotonic with NaCl. Methemoglobin concentration and red cell hexose monophosphate shunt (HMPS) metabolism (measured by the conversion of ^{14}C-labeled glucose to $^{14}CO_2$) were assayed by known techniques (*5, 6*). Chlorine and chloramine were assayed by the *o*-tolidine method (*7*) which detects total chlorine (that is, OCl^-, HOCl, NH_2Cl, $NHCl_2$, and NCl_3). Reference values for the *o*-tolidine test were obtained by titration of standard solutions of calcium or sodium hypochlorite with iodine and thiosulfate (*8*). In most of the experiments, chlorinated water was prepared by adding previously assayed sodium hypochlorite alone or with ammonium hydroxide (to form chloramines) in a 1 : 2 molar ratio to distilled water containing 0.15*M* NaCl (*9*). During hemodialysis, relatively small volumes of blood are exposed to large volumes of dialyzing solution. Therefore, in most experiments, 0.5 ml of packed, saline-washed red cells were added to 50 ml of test water made isotonic through the addition of dry NaCl.

Hemodialyzed patients from three University of Minnesota hospitals were studied. Significant methemoglobinemia (> 5 percent) and Heinz body inclusions in red cells were regularly observed in patients in two hospitals, but not in a third. Unpurified tap water and RO water were utilized for dialysis baths in the former two, whereas charcoal-filtered water was used in the third. Analogously, red cells briefly incubated in Minneapolis tap water before or after

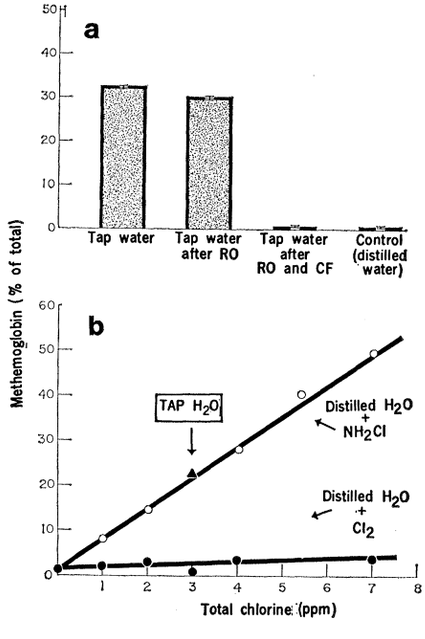

Fig. 1. (a) Production of methemoglobin in washed red cells by water treated in various ways. One volume of red cells was incubated for 15 minutes at 37°C with 100 volumes of isotonic NaCl solution made from the indicated types of water. Red cells were subsequently packed and assayed for methemoglobin. Means ± 1 standard deviation are shown in the center of each bar. (b) Effects of chlorination and chloramination on methemoglobin production in washed red cells. Note the lack of effect of chlorine (added as sodium hypochlorite) relative to that of chloramines. Chloramines were made by mixing molar concentrations of sodium hypochlorite and ammonium hydroxide (1 : 2). The major product of this mixture (at our incubation *p*H of about 7.6) is assumed to be in the form of NH_2Cl. Methemoglobin accumulation in red cells incubated in Minneapolis tap water is depicted by the black triangle and coincides with the value obtained with chloraminated water of the same total chlorine content. Conditions of incubation are the same as in (a).

RO purification accumulated large amounts of methemoglobin. However, with charcoal filtration of the RO water the oxidant effect disappeared (Fig. 1). Oxidation of red cells by RO water was stoichiometric, more methemoglobin being formed when a given volume of red cells was exposed to increasing amounts of water. After incubation and removal of red cells by centrifugation, the supernatant was no longer capable of oxidizing newly added red cells, an indication that the oxidant in RO water was consumed.

The oxidant was volatile and was removed from the RO water by exposure to vaccum or by boiling. The possibility that the oxidant was chlorine or a chlorine compound was suggested by: (i) its volatility; (ii) the fact that it occurred in both city water and in water purified by reverse osmosis, which does not remove gaseous contaminants; and (iii) its noticeable odor. However, simple chlorination of isotonic distilled water with either gaseous chlorine or sodium hypochlorite failed to reproduce the oxidant effects of tap water as measured by methemoglobin accumulation in suspended red cells (Fig. 1). Because most urban water supplies are chlorinated with a combination of chlorine and ammonia to form chloramines, we treated distilled water similarly. This water exactly reproduced the oxidant effect of tap or RO water containing equivalent concentrations of assayable chlorine (Fig. 1). The results indicate that, in the water under investigation, chloramines are probably the oxidants that caused the hemolytic anemia in our dialyzed patients.

Serial observation of several patients undergoing dialysis suggested that the red cell oxidant damage was cumulative over several periods of dialysis. Investigation revealed that chloramines not only cause direct oxidant damage to red cells, but inhibit the metabolic pathway used by these cells to prevent and repair such damage. The HMPS, through generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH), protects the red cell from oxidant damage. When this pathway is defective, as it is in inherited glucose-6-phosphate dehydrogenase deficiency, red cells become vulnerable to damage from oxidant compounds such as primaquine (*10*). The HMPS metabolism, as measured by $^{14}CO_2$ evolution from [1-^{14}C]glucose and [2-^{14}C]-glucose, was inhibited in red cells which had been previously incubated in isotonic solutions made from tap or RO water. This inhibition was relieved if RO water was first filtered through charcoal to remove chloramines (Fig. 2).

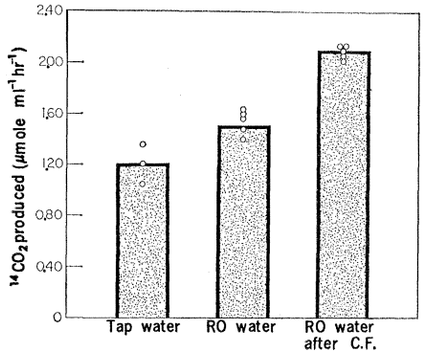

Fig. 2. Inhibited hexose monophosphate shunt metabolism in red cells suspended in urban waters. To duplicate flasks containing [1-^{14}C]glucose and [2-^{14}C]glucose (1 μc) were added washed red cell suspensions of the same hematocrit prepared in isotonic phosphate-buffered saline [12 m*M* $(PO_4)^{-3}$; *p*H 7.4] using various waters. Each value shown is the sum of $^{14}CO_2$ evolved from both flasks in the presence of methylene blue (*6*).

Our investigations indicate that chloramines may cause marked methemoglobinemia and hemolysis in hemodialyzed patients. A similar process may underlie the lethal effects of chlorine compounds which have been reported in different species of freshwater fish (*11*). We have some evidence that tends to support this possibility. Four representatives of *Pimephales promelas* (fathead minnow), weighing about 1 g each, were exposed to water containing chloramines [1.5 parts per million (ppm)]. Fish exposed to the chloramines were removed from the water just as they began to lose their righting reflex. Blood was taken from each fish by cutting off the tail and immersing the fish in distilled water containing 1 m*M* EDTA. The hemolyzates were pooled, and the methemoglobin content was analyzed. Fish exposed to chloramines for about 40 minutes averaged 32 percent methemoglobin (controls were less than 3 percent).

Chloramine-containing dialysis water presents a severe threat to the uremic patients undergoing dialysis. Since patients with uremia characteristically manifest inefficient erythropoiesis, severe anemia may be precipitated by agents that shorten the survival time of red cells. Chloramines may decrease survival (i) by oxidatively denaturing hemoglobin into Heinz bodies and (ii) by reducing the ability of red cells to repair this damage. The latter may potentiate the intrinsic defect of HMPS metabolism in uremic red cells (*12*). Many uremic patients are candidates for renal transplantation, in which the probability of rejection is increased if isoimmunity is stimulated by previous transfusions. It seems especially important, therefore, that chloramine-induced hemolytic anemia be avoided in hemodialysis units by appropriate treatment of water. Purification of water by RO alone is not sufficient. We have evidence that addition of ascorbic acid to dialysis baths detoxifies chloramines as efficiently as charcoal filtration (*13*). Both modes of water treatment eliminate all detectable chlorine compounds and completely normalize red cell survival in dialyzed uremics.

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