Inactivation of the Virus of Infectious Hepatitis in Drinking Water*

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IN previous reports, the transmission of a virus of infectious (epidemic) hepatitis by drinking water to most of the residents of a summer camp for boys and girls [1] and the results of preliminary experiments on the disinfection of water containing this virus were described.[2] Although the epidemiological and experimental data afforded adequate evidence that the virus responsible for the summer camp epidemic was waterborne, circumstances prevented an adequate investigation of the suspected source (a cess pool) of contamination of the water supply (a deep well) at the time of the epidemic. Subsequently, a careful sanitary investigation of the relationship of the cess pool to the well provided evidence, described elsewhere,[3] that the water supply could have been contaminated with sewage material from the cess pool suspected at the time of the epidemic. This finding thus further strengthened the previous conclusions [1] based on other epidemiological and experimental observations.

The "hepatitis viruses" pass through filters which retain bacteria,[1, 4] and they have survived heating at 56° C. for at least ½ hour,[4] have remained active for several years in materials kept in the frozen state [5] or in the liquid state at 4° C.,[6] and one in desiccated yellow fever vaccine was found to be active after storage for at least 1 year at room temperature.[7] "Hepatitis viruses" in plasma or serum have remained active in the presence of merthiolate in a concentration of 1 to 2,000 [8] and in the presence of a mixture of equal parts of phenol and ether added to a concentration of 0.5 per cent.[9] The available evidence concerning the properties of these viruses thus indicates that they are resistant to certain procedures which eliminate or destroy bacteria and suggests that certain methods of water disinfection known to be adequate for bacterial intestinal pathogens may not be adequate for the more resistant viruses.

Preliminary studies [2] have shown that treatment of heavily contaminated

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water with sufficient chlorine to produce a total chlorine residual of 1 p.p.m. after 30 minutes' contact (Lyster bag technique used for emergency disinfection of drinking water in the field) did not inactivate or attenuate a virus of infectious (epidemic) hepatitis that had been added to the water (water specimen 2, Table 2). Treatment of similarly contaminated water by the "breakpoint" chlorination technique (followed by dechlorination) apparently did inactivate this virus,[2] as none of the 5 volunteers ingesting such treated water developed clinical manifestations of the disease (water specimen 5, Table 2). Four of the 5 men, however, developed positive cephalin cholesterol flocculation tests between the 42nd and 67th days after ingestion of this water. Subsequently, the 5 men were inoculated with the untreated active virus and 4 of the 5 developed typical acute infectious hepatitis after incubation periods of 3 to 4 weeks. As other studies have indicated that inapparent infections unaccompanied by any laboratory evidence of hepatic disturbance may be followed by resistance to reinfection with the same virus,[5] there is some doubt as to whether the positive cephalin cholesterol flocculation tests following the ingestion of the treated water were due to inapparent hepatitis. Because the interpretation of the positive cephalin cholesterol flocculation tests is not clear, however, and as they apparently were not technical in origin, the results indicate that "breakpoint chlorination" of this heavily contaminated water either completely inactivated the hepatitis virus or at least inactivated it to the extent that it was not able to induce clinically apparent hepatitis or resistance to reinfection.

Finally it was found[2] that treatment of contaminated water with sodium carbonate, aluminum sulfate, and activated carbon did not completely remove or inactivate the hepatitis virus although some decrease in the concentration of the virus and possibly some decrease in its virulence apparently resulted (water specimen 4, Table 2).

As these initial studies suggested that the hepatitis viruses might be more resistant to chlorine than the pathogenic bacteria commonly encountered in water, and the present methods of water disinfection are based on their effectiveness in controlling the bacterial pathogens, further studies of their effect on the hepatitis virus appeared desirable. The present report deals with the results of additional studies on this problem, particularly with those obtained with different dosages of chlorine applied after preliminary coagulation and filtration.

MATERIALS AND METHODS

Preparation of Contaminated Water Specimens (Raw Specimens) — The method of preparation of the raw specimens was the same as that used in the previous experiments.[2] Briefly, specimens of distilled water were contaminated with the virus of infectious (epidemic) hepatitis described in previous reports[1, 2, 5] by adding to each a similar quantity (varying from 40 to 50 parts of feces per million parts of water in the different experiments) of a feces suspension known to contain that virus. Approximately 2 ml. of a suspension of *Escherichia coli* also were added to each specimen for the purpose of facilitating a study of the effect of the treatment procedures on the bacterial counts. The raw specimens then were allowed to stand for a period of 24 hours at room temperature before further treatment was applied.

Water Specimen 6 (Control)—The raw specimen was siphoned off and passed through a pad composed of 4 layers each of sterile cotton and gauze, the object being only to remove any solid particles of feces that remained. This specimen, which was not sub-

jected to further treatment, was used as a control for specimens 7, 8, and 9, the results obtained with specimen 6 indicating those that would have been expected from specimens 7, 8, and 9 prior to treatment.

Water Specimen 7—The raw specimen was coagulated by adding 2.0 grains of sodium carbonate per gallon of water and 4.0 grains of aluminum sulfate per gallon of water. After stirring gently for 15 minutes, which resulted in the production of a heavy, promptly settling "floc," the specimen was allowed to stand for one hour without disturbance. During this period, the "floc" settled. The clear, colorless supernatant was siphoned with sterile apparatus through a sterile gauze and cotton pad into a sterile bottle. The water then was filtered through a model diatomite filter (employing a diatomaceous silica filter coat) of the type developed during the war for use by the United States Army in the field and described in detail elsewhere.[3] The filtrate was received in a sterile bottle. A dosage of 3.25 p.p.m. of freshly prepared chlorine solution was applied to the filtered water and after 30 minutes' contact the total chlorine residual (measured by the starch-potassium iodide method, titration with sodium thiosulfate) was found to be 1.17 p.p.m. Using the orthotolidine test (color comparator method), the residual chlorine read approximately 1.0 p.p.m. An orthotolidine arsenite test [10] indicated a residual free chlorine content slightly in excess of 0.4 p.p.m. Forty-two minutes after the chlorine had been added, the specimen was completely dechlorinated with sodium sulfite, this representing the final step in in its treatment.

Water Specimen 8—Except for the addition of 25 p.p.m. of activated carbon to the raw specimen simultaneously with the sodium carbonate and aluminum sulfate and a different chlorine dosage, specimen 8 was treated exactly as specimen 7. A dosage of 7.5 p.p.m. of chlorine was applied and resulted in a 30 minute total residual chlorine content of 5.2 p.p.m. The specimen was dechlorinated 36 minutes after the chlorine had been added, this completing its treatment.

Water Specimen 9—The specimen was treated exactly as specimen 8 except for the dosage of chlorine applied. A dosage of 15 p.p.m. of chlorine was used and the 30 minute total chlorine residual was 11.3 p.p.m. The specimen was dechlorinated 34 minutes after the chlorine had been added, this completing the treatment.

Water Specimen 10 (Control)—This differed from the control specimen 6 only in that the quantity of the feces suspension added to the distilled water in preparing the raw specimen was slightly greater for specimen 10. Raw specimen 10 was strained in the same fashion as specimen 6 and was used without further treatment as a control for specimens 11 and 12.

Water Specimens 11 and 12—A raw specimen identical with, but twice the volume of, specimen 10 was coagulated, allowed to settle, and then filtered in the same manner as specimen 7. One-half of the filtrate was removed to a separate sterile bottle. This constituted *specimen 11* which received no additional treatment. The other half (*specimen 12*) then was chlorinated, a dosage of 3.25 p.p.m. being applied. The 30 minute total chlorine residual was 2.0 p.p.m. by the starch potassium iodide method and approximately 1.5 p.p.m. by the orthotolidine test, 0.4 to 0.45 p.p.m. of this being in the form of free chlorine as indicated by the orthotolidine-arsenite test. Specimen 12 was dechlorinated by the end of 36 minutes, this completing the treatment. Specimens 11 and 12 thus both were coagulated, allowed to settle, and the clear supernatant filtered through a diatomite filter. Specimen 12, in addition, was chlorinated.

Studies on Water Specimens—Chemical and bacteriological analyses of each of the specimens were conducted by the methods described previously.[2] The studies for the presence of, or the effect of the treatment procedure on, the hepatitis virus were conducted in human volunteers in the same manner and under the same controlled conditions as those described previously.[2] The volunteers all were under 33 years of age and had no previous history of jaundice, no previous experimental inoculations, and no current evidence of hepatic disturbance detectable by history, physical examination, or laboratory tests. Isolation of the volunteers by groups was carried out as in the previous experiments.[2] All needles and syringes used on the volunteers for any purpose in these studies were sterilized by autoclaving for 20 to 30 minutes before each use. Following ingestion of the water specimens, the volunteers were under continuous observation and study for a minimum period of 6 months.

TABLE 1

Water Specimen	pH	Chemical Analyses: Total Solids Tot.	Total Solids Org.	Sus. Solids Tot.	Sus. Solids Org.	Nitrogen: Total	Albuminoid	Ammonia	Nitrite	Nitrate	Bacteriological Analyses: Tests for E. coli Presumptive	Confirmed	Bacteria per ml. (37° C., 24 hrs.)
6 (Control)	6.4	60	38	20	13	0.92	0.65	0.043	0.005	0.02	+	+	1,225,000,000
7	6.8	82	6	3	2	0.33	0.17	0.010	0.001	0.01	—	—	—
8	6.8	86	14	8	4	0.25	0.15	0.009	0.001	0.01	—	—	—
9	6.9	98	9	7	3	0.26	0.18	0.009	nil	0.01	—	—	—
10 (Control)	6.9	45	..	23	22	....	1.44	0.05	0.013	3.0	+	+	8,000,000,000
11	6.7	87	3	0.2	0.2	....	0.38	0.167	0.011	1.9	+	+	173,520,000
12	7.3	119	10	0.2	0.2	....	0.34	0.285	0.003	1.6	—	—	10

Summary of results of chemical and bacteriological studies made on Water Specimens 6 to 12. See Table 2 for data on treatment of these specimens. All chemical results, except pH, are expressed in parts per million. pH measured at 25° C. "Tot.," "Sus.," "Org." represent total, suspended, and organic respectively.

RESULTS

A detailed presentation of the results of the chemical and bacteriological analyses of the various water specimens is not within the scope of this paper. These results, summarized in Table 1, are considered more fully elsewhere.[3]

Because of the limitations imposed by the number of available volunteers, it was necessary to carry out the present studies in two parts. Water specimens 6, 7, 8, and 9 were prepared simultaneously and ingested by different groups of volunteers (groups VI, VII, VIII, and IX) in August, 1945. Specimens 10, 11, and 12 were prepared simultaneously and ingested by different groups of volunteers (groups X, XI, and XII) in November, 1945. The conduct of the two parts of the experiments at different times required the use of a control group each time (groups VI and X, water specimens 6 and 10 respectively).

The effect of the various treatment procedures on the hepatitis virus, as indicated by comparison of the results in the volunteers after ingestion of the treated and untreated (control) water specimens were as follows (Table 2):

Water Specimen 6 (untreated; control)—Each of the 5 volunteers ingested approximately 2¾ liters of this specimen over a period of 24 hours. Three of the 5 developed typical infectious hepatitis after incubation periods of 23, 24, and 27 days respectively. One other subject developed fever, abdominal pain, anorexia, nausea, and malaise only 8 days after inoculation. Because of the possibility that the unusually early onset, if the symptoms were due to hepatitis, might be indicative of unusual susceptibility resulting in an especially severe attack, he was treated intensively (intravenous plasma, glucose, and methionine) beginning on the first day of his illness. Prompt recovery followed without the development of jaundice or significant laboratory evidence of hepatic disturbance. Excluding this case, in which the diagnosis was uncertain, the incidence of unquestionable hepatitis in this control group thus was at least 60 per cent.

Water Specimen 7 (coagulated, filtered, 3.25 p.p.m. chlorine dosage)—None of the 5 volunteers, each of whom ingested approximately 2¾ liters of this specimen, developed clinical or laboratory manifestations of hepatitis or other illness during a 6 month period of observation.

Water Specimen 8 (coagulated, activated carbon, filtered, 7.5 p.p.m. chlorine dosage)—None of the 5 volunteers, each of whom ingested approximately 2¾ liters of this specimen, developed clinical or laboratory manifestations of hepatitis or other illness during a 6 month period of observation.

Water Specimen 9 (coagulated, activated

TABLE 2

Treatment of Water Specimens				Chlorine (p.p.m.)			Results in Volunteers		
					30′ Residual				
Specimen	Coagulated	Act. Carbon	Filtered	Dose	Total	Free	No. Inoc.	Hepatitis	Incubation Period (Days)
1 (Control)	—	—	—	—	—	—	5	2	22, 24
2	—	—	—	2.5	1.08	N.D.	5	2	23, 28
3 (Control)	—	—	—	—	—	—	5	4	19, 19, 19, 22
4	+	+	—	—	—	—	5	2	33, 37
5	—	—	—	25	15	N.D.	5	0	—
6 (Control)	—	—	—	—	—	—	5	3	23, 24, 27
7	+	—	+	3.25	1.1	0.4	5	0	—
8	+	+	+	7.5	5.2	N.D.	5	0	—
9	+	+	+	15	11.3	N.D.	5	0	—
10 (Control)	—	—	—	—	—	—	6	5	18, 20, 21, 24, 27
11	+	—	+	—	—	—	7	3	27, 30, 31
12	+	—	+	3.25	2.0	0.4	7	0	—

Effect of various procedures employed in the disinfection of drinking water on a virus of infectious (epidemic) hepatitis as indicated by the incidence of hepatitis in volunteers after ingestion of such treated water. The data on Specimens 1 and 2 and on Specimens 3, 4, and 5 are from two earlier experiments (cf. 2). These data have been included to facilitate comparison with the present results. "Coagulated" refers to the treatment of water with aluminum sulfate and sodium carbonate. "Act. Carbon" indicates the use of activated carbon as an adsorbent. "Filtered" refers to filtration through a model diatomite filter with a diatomaceous silica filter coat; "p.p.m." indicates parts of chlorine per million parts of water. "N.D." represents "not determined." "Hepatitis" refers to the number of volunteers who developed unquestionable infectious hepatitis following ingestion of the various water specimens ("inoc."—inoculation).

carbon, filtered, 25 p.p.m. chlorine dosage)—None of the 5 volunteers, each of whom ingested approximately 2¾ liters of this specimen, developed clinical or laboratory manifestations of hepatitis or other illness during the 6 month period of observation.

Water Specimen 10 (untreated; control)—Of the 6 volunteers who each ingested approximately 2¾ liters of this untreated specimen, 5 developed infectious hepatitis after incubation periods of 18, 20, 21, 24, and 27 days respectively.

Water Specimen 11 (coagulated, filtered, no chlorine)—Of the 7 men who each ingested approximately 2¾ liters of this specimen, 3 developed infectious hepatitis after incubation periods of 27, 30, and 30 days respectively.

Water Specimen 12 (coagulated, filtered, 3.25 p.p.m. chlorine)—None of the 7 volunteers, each of whom ingested approximately 2¾ liters of this specimen, developed clinical or laboratory manifestations of hepatitis or other illness during the 6 month period of observation.

COMMENT

The results show that the hepatitis virus was inactivated in heavily contaminated water treated by coagulation, filtration, and the application of sufficient chlorine to provide, after 30 minutes' contact, total and free residual chlorine concentrations of 1.1 and 0.4 p.p.m. respectively. The effect of the coagulation, settling, and filtration alone (no chlorine) was shown by the results with specimens 10 and 11. The ingestion of coagulated, settled, and filtered water was followed by a 43 per cent incidence of hepatitis. This represented a 40 per cent decrease in the

incidence as compared with that observed in the control group following ingestion of the untreated contaminated water. In a previous experiment,[2] coagulation and settling alone (water specimen 4, Table 2) also decreased the incidence of hepatitis by 40 per cent as compared with that in the group receiving untreated water (specimen 3). Although the groups are too small for the results to be statistically significant, the occurrence of a decrease in incidence in two consecutive similar experiments suggests that this part of the treatment had some effect on the virus. This effect probably was chiefly the result of a decrease in the concentration of the virus as the absence of any apparent difference in the severity of the disease resulting from specimen 11, as compared with that in the control group (specimen 10), suggested that the virulence of the virus was not appreciably influenced. It is of interest, however, that the *minimum* incubation period in those who received the coagulated, settled, and filtered water was 27 days whereas the *maximum* incubation period in the control group (water specimen 10) was 27 days (Table 2). This also was observed in the previous similar experiment, the minimum incubation period in those receiving coagulated and settled (but not filtered) water (water specimen 4, Table 2) being 33 days as compared with a maximum incubation period of 22 days in the control group (water specimen 3). Combining these two similar groups who received coagulated and settled (specimen 4) or coagulated, settled, and filtered (specimen 11) water and the two respective control groups (specimens 3 and 10), the mean incubation period for the former was 31.6 days (5 cases) and that for the latter was 21 days (9 cases). The minimum incubation period of 27 days in the former group was observed in only 1 case, the period in the other 4 ranging from 30 to 37 days. The maximum incubation period in the combined control groups was 27 days, the period in 7 of the 9 cases ranging from 19 to 22 days. In spite of the small size of the groups involved, the difference in the mean incubation periods is statistically significant and shows that this type of water treatment (regardless of whether the effect was due to decrease in concentration of the virus, its virulence, or both) resulted in an appreciable increase in the length of the incubation period. Although not demonstrated conclusively, the data suggest that this was due chiefly to the coagulation and settling part of the procedure, the results with water that only had been coagulated and settled being similar to those with water which had been coagulated, settled, and filtered. The failure of this type of filter to remove the hepatitis virus, which passes through Seitz and other bacteria retaining filters, is not surprising as the diatomite filter did not retain bacteria (Table 1, bacteriological studies), although the bacterial count apparently was decreased by such filtration.

It is obvious, therefore, that the complete control of the hepatitis virus (and probably other infectious agents) in drinking water depends, in the final analysis, almost entirely on the disinfectant used and its proper application. These studies have shown that the application of sufficient chlorine to provide, after 30 minutes' contact, a total chlorine residual of approximately 1 p.p.m. and a free chlorine residual of 0.4 p.p.m. was capable of inactivating the hepatitis virus in heavily contaminated water when the water had been treated previously by coagulation, settling, and filtration. The minimal effective dose of chlorine under these conditions was not determined due to the lack of volunteers and the more urgent need of determining a reliable method for use by the army in the field.

Although the lowest dosage of chlorine applied, in these experiments, to coagulated, settled, and filtered water was effective in inactivating the hepatitis virus, the resulting total residual chlorine concentration was greater than that ordinarily required in municipal water practice. Conclusions regarding the adequacy, in respect to the hepatitis virus, of smaller doses of chlorine must await the determination of the minimal effective dose in coagulated and filtered water. It is significant however, that the 30 minute residual total chlorine concentration of 1 p.p.m., shown to be effective in coagulated, settled, and filtered water, failed to inactivate or attenuate this virus in previously untreated water.[2] The difference between the effect of the same total residual chlorine in untreated water as compared with that in previously coagulated, settled, and filtered water may be due in part to a conversion of most of the free chlorine to chloramines, which are known to be less active disinfectants than is free chlorine, by the greater quantity of unoxidized organic material in the former. This indicates the importance of proper pretreatment (coagulation, settling, filtration) of water, particularly if polluted water is the original source, in order to decrease the quantity of unoxidized organic materials before chlorine is applied.

These observations permit some speculation about a possible source of origin of some small outbreaks of infectious hepatitis and of some of the apparently sporadic cases. It is emphasized that the following observations are entirely theoretical and not based on factual evidence. As the virus of infectious hepatitis is excreted in the feces of infected persons and some communities use polluted streams as the original source of their water supply, it appears possible that any break or inadequacy in the numerous steps concerned in the preparation of such water for human consumption might permit the survival of small quantities of hepatitis virus. Considering some of the many factors that may be involved—polluted water as the original source, probable wide variation in the degree of pollution, the final dependence of adequate disinfection on the quantity and efficiency of the disinfectant (chlorine) used, this in turn dependent on the composition of the water, variation of the composition with the degree of pollution and the efficiency of the coagulation and filtration procedures, the limitation imposed by palatability (taste, odor, etc.) on the quantity of disinfectant that can be used, the resistance of the hepatitis virus (and possibly some other viruses) to procedures which ordinarily destroy or eliminate bacteria, and the necessity of supplying large quantities of water continuously without interruption—it would not be surprising if small quantities of some of the more resistant infectious agents occasionally survived. This could account for some of the occasional small outbreaks of hepatitis or for apparently sporadic cases at widely separated points served by the same source of water.

SUMMARY AND CONCLUSIONS

1. The effect of certain procedures commonly employed in the disinfection of drinking water on a virus of infectious (epidemic) hepatitis has been investigated with the following results:

a. Coagulation, settling, and filtration (diatomite filter) of contaminated water did not eliminate or inactivate the hepatitis virus as the disease developed in 40 per cent of the volunteers who ingested such treated water. However, this treatment resulted in a prolongation of the incubation period and a 40 per cent decrease in the incidence as compared with that in the control group.

b. The application to such water (previously coagulated, settled, and filtered) of sufficient chlorine to provide, after 30 minutes' contact, total and free residual chlorine concentrations of 1.1 and 0.4

p.p.m. respectively apparently was adequate to inactivate the hepatitis virus under the conditions of this experiment. However, the same 30 minute residual total chlorine concentration (1 p.p.m.) in contaminated water that had not been pretreated by coagulation, settling, and filtration did not inactivate the hepatitis virus.

2. The complete control of the hepatitis virus (and probably other infectious agents) in drinking water depends almost entirely on the disinfectant as the virus is not eliminated or inactivated by preliminary coagulation, settling, and filtration of the water. The efficiency of the disinfectant varies with the character of the water and the resistance of the infectious agent concerned. Dosages of chlorine that are adequate for inactivation of the bacterial pathogens occurring in drinking water may not be adequate for the hepatitis virus, particularly if the water has a high content of unoxidized organic material. Conclusions regarding the adequacy, in respect to the hepatitis virus, of the dosages of chlorine ordinarily applied for the disinfection of water must await the determination of the minimal effective dose of chlorine in coagulated, settled, and filtered water.

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