

The second part of this investigation deals with the efficiency of a two-stage flocculation process for removal of viruses and native bacteria in raw river water.

REMOVAL OF COXSACKIE AND BACTERIAL VIRUSES IN WATER BY FLOCCULATION

II. Removal of Coxsackie and Bacterial Viruses and the Native Bacteria in Raw Ohio River Water by Flocculation with Aluminum Sulfate and Ferric Chloride

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IN PART I of this investigation¹ a study was made of the relative efficiency of alum and ferric chloride for the removal of Coxsackie and bacterial viruses by flocculation in chemically defined water and the effects of coagulant dosage, buffering system and pH, and rate of stirring on the removal efficiency of alum were described. In the present investigation an examination was made of the effectiveness of flocculation processes as practiced in water treatment plants for removing viruses and native bacteria in raw Ohio River water. Some attention was also devoted to determining the critical contact time between microorganisms and coagulant in water by alum flocculation and to studying the interference effect of gum arabic on the removal of viruses and bacteria in water by flocculation.

Materials and Procedures

Materials

Strains of Coxsackie and bacterial viruses were used as previously described.¹ By use of purified Coxsackie

suspensions and dilute bacterial virus suspensions, insignificant amounts of organic materials were added to the experimental waters. Suspension of *Escherichia coli* prepared by washing slant cultures into sterile distilled water were employed in determining the critical time for the removal of bacteria by flocculation. The suckling mice and laboratory supplies were the same as those used in the preceding study. Standard media were used in determining the most probable numbers of coliform and total bacteria and for growing *E. coli*.

Procedures

Purification of Coxsackie Virus: The frozen, clarified suspension of Coxsackie virus used in the preceding study¹ was thawed and frozen two to three times. The suspension was then diluted in sterile distilled water containing 5 ppm SiO_2 and 3 millimols Na_2CO_3 and flocculated with 360 ppm $\text{Al}_2(\text{SO}_4)_3$. The floc was resuspended in 0.1 molar bicarbonate solution of pH 8.5–9.0 and was allowed to stand with frequent agitation

to facilitate separation of virus from the floc. After centrifuging, the supernatant containing the purified virus was removed and its pH adjusted to 7.0–7.3 with 1 N HCl (slight pink to phenol red) and its volume made up to that of the original suspension. The purified suspension was again frozen at -70°C until used. With this purification procedure a drop of 30–50 per cent of the infective titer and a removal of 96.7–99.7 per cent of the organic nitrogen were observed, i.e., the LD_{50} dropped from about 3 million to 1.5–2.0 million per 0.02 ml and the organic nitrogen from 4,000 to 1.5–15 mg/ml.

Collection of Raw River Water Samples: Most of the raw river water samples were collected from a tap at the Cincinnati Water Purification Plant, with the exception of a few water samples which were collected at the public landing located about 10 miles downstream from the plant intake.

Turbidity of the river water fluctuated from a few ppm to a high of over 600 ppm. Samples having turbidity readings over 400 ppm were found to have such high coagulant demands that several times the ordinary dosage was required for a good floc formation. Hence, all the samples in the present study showed turbidities from 16 to 255 ppm and were used in experiments within a few hours after collection.

Test Procedure: For the two-stage process, the procedure used in the first part was that previously described for the one-stage process, with alum the coagulant. In the second stage, the supernatant obtained in the first was measured into another flocculation beaker and dosed with a calculated amount of the 1 per cent FeCl_3 solution and sufficient 0.1 per cent CaO solution to produce a final pH in the range of 7.3–8.4 as practiced in waterworks. The treated water was stirred for 30 minutes at 81 rpm followed by 90 minutes of standing before sampling the supernatant for de-

termination of microorganism concentration.

Determination of Concentrations of the Coxsackie and Bacterial Viruses in Samples of Settled and Control Water: The procedures for determining the bacterial and Coxsackie virus concentrations were identical to those described in the preceding report except that penicillin and streptomycin were used in inocula for mice in order to suppress possible bacterial infection.

Again, at least 18 mice were used for each dilution and total numbers were comparable to those of the preceding study.¹

Determination of the Most Probable Number of Coliform and Total Bacteria in Samples of Settled and Control Water: Tenfold dilutions in sterile buffered distilled water were prepared from all samples of the settled water and the control. The coliform MPN (most probable number) was determined in accordance with the procedure prescribed in Standard Methods² and the total bacteria MPN was estimated by referring the number of positive tubes (indicated by visible bacterial growth) in each of three suitable consecutive dilutions to the MPN table.²

Experimental Data

Removal of Viruses and Native Bacteria in Raw Ohio River Water by Two-Stage Flocculation at 5°C – 25°C

Using 25 ppm of $\text{Al}_2(\text{SO}_4)_3$ in the first stage and the same amount of FeCl_3 in the second stage, a number of determinations were made to ascertain the virus and bacteria-removal efficiency of the two-stage process at 5°C , 15°C , and 25°C . The percentage removal was computed for all four categories of microorganisms and is shown in Table 1, together with observations on turbidity, pH readings, and floc formation.

Table 1 shows that the percentage removal of Coxsackie virus in the first-

Table 1—Efficiency of Two-Stage Flocculation in Removing Viruses and Native Bacteria in Raw Ohio River Water at Varying Temperatures

1st stage: 25 ppm $\text{Al}_2(\text{SO}_4)_3$
2nd stage: 25 ppm FeCl_3

Temperature C	Initial Turbidity (ppm)	Stage of Flocculation	Per cent Removal				Final Turbidity (ppm)	Final pH	Floc Formation
			Coxsackie Virus	Bacterial Virus	Coliform Bacteria	Total Bacteria			
25	16-240	1st	98.6	94.8	99.8	99.8	1-5	6.7-7.3	Very good
	1-5	2nd	93.8	98.1	93.8	94.8	0.1	7.3-7.8	Good
	Combined	99.9	99.9	99.99	99.99	0.1
15	140-255	1st	94.8	88.6	94.4	99.3	1-5	6.7-7.4	Very good
	1-5	2nd	92.1	89.9	82.4	78.0	0.1	7.3-7.7	Good
	Combined	99.6	98.6	99.9	99.8	0.1
5	40-135	1st	95.9	84.4	98.8	98.7	1-5	6.7-7.4	Very good
	1-5	2nd	93.8	94.0	61.6	88.4	0.1-1	7.3-7.7	Good
	Combined	99.6	99.1	99.95	99.8	0.1-1

stage flocculation was slightly but consistently higher than that of bacterial virus at all three temperatures. This observation was in contrast to that observed with water of known chemical content in the preceding study. This reversal of removal pattern was not attributable to the use of purified Coxsackie suspension. Since it has been reported that calcium and magnesium ions become attached to bacterial virus³⁻⁵ and since raw Ohio River water contained calcium ion (23.6-53.6 ppm) and magnesium ion (6.3-13.6 ppm) during the study period, it appears possible that the presence of these metal ions slows down the formation of the aluminum-bacterial virus complex.

The removal of both viruses attained at all three temperatures by 25 ppm of $\text{Al}_2(\text{SO}_4)_3$ in the first stage compared favorably with that attained by 40 ppm in water of known chemical content in the preceding study. It was also noted (Table 1) that the floc formation with the 25 ppm $\text{Al}_2(\text{SO}_4)_3$ was good at all three temperatures, although it was slower at 5° C than at 15° C and 25° C.

The percentage removal of Coxsackie and bacterial virus obtained with 25 ppm of FeCl_3 in the second-stage flocculation was somewhat irregular but, as a whole, was comparable to that obtained with 25 ppm of alum in the first stage. The removal of the bacterial virus in the second stage was slightly but uniformly lower than that obtained with 20 ppm FeCl_3 . It was shown in the preceding study that the ferric compound was definitely more efficient than alum in removing bacterial virus. Since the supernatant from the first stage was relatively free from turbidity and the floc formation in this stage was better than in the second stage, the slightly lower virus-removal efficiency of the FeCl_3 observed here could be due to the lack of sufficient colloidal matter to facilitate as good formation of the iron-virus complexes as in the raw water. The addition of sufficient CaO solution to give a final calcium ion concentration of 60-62 ppm in the second stage, together with the natural calcium and magnesium ions in the river water, again may have contributed to the lower

removal of the bacterial virus from the river water than was observed in the preceding study.

At all three temperatures the removals of total bacteria and of coliforms in the first stage were consistently higher than the virus removals. This bacteria-virus removal ratio is in agreement with that reported by Gilcreas and Kelly,⁶ although the per cent removal was decidedly higher in this study than that obtained by them.

Lowering of the temperature from 25° C to 15° C and 5° C lowered the per cent removal of the Coxsackie virus and bacteria very slightly and somewhat more significantly lowered the per cent removal of the bacterial virus in the first stage. In the second stage, the lowering of the temperature did not materially affect the removal of viruses but lowered the removal of the bacteria more than in the first stage. Since the supernatant from the first stage carried, after the addition of CaO, about 100 ppm of calcium ion and about 10 ppm magnesium ion, and since the presence of 100–200 ppm of calcium or magnesium ion in water has been found to raise the isoelectric point of *E. coli* cells from the acid to the basic side of the pH range (unpublished data of S.L.C.), this reduced bacteria removal in the second stage could be explained again by the interfering effect of these bivalent cations in slowing down the formation of the iron-bacteria complexes, especially at lower temperatures.

The percentage removal of all four categories of organisms was quite high. The removal of bacterial virus and bacteria was slightly but consistently higher at 25° C than at 15° C and 5° C, and the removal of the bacteria was also slightly but consistently higher than that of the viruses. These results clearly indicate that a two-stage flocculation process employing 25 ppm each of these two coagulants removed 99.6–99.9 per cent of the Coxsackie virus at 5° C–25° C,

and 99.99 and 99.8–99.95 per cent of bacteria at 25° C and 5° C–15° C, respectively.

There was no apparent relationship shown in Table 1 between the turbidity of raw river water and the removal of microorganisms. It appears, therefore, that at an alum dosage of 25 ppm and turbidity levels under 240 ppm, turbidity itself is not a satisfactory criterion for judging the efficiency of the process in removing viruses and bacteria.

Effect of Coagulant Dosage in Removing Viruses and Bacteria in Raw Ohio River Water by Flocculation

The two-stage process was repeated at 25° C, with the dosage of alum and ferric chloride reduced to 15 ppm. Table 2 contains a summary of the virus and bacteria removal results, together with turbidity and pH values, and some observations on floc formation. For the purpose of comparison, the results obtained with 25 ppm of these coagulants at 25° C (Table 1) are reproduced in Table 2.

Removal of the Coxsackie virus by 15 ppm of aluminum sulfate was only slightly lower, but removal of the bacterial virus and the bacteria, especially the latter, was distinctly lower than that afforded by 25 ppm of this coagulant. This reduced removal of bacterial virus and bacteria may again be due to the interfering effect of calcium and magnesium ions in the raw river water, which became significant when the alum dosage was lowered from 25 to 15 ppm. It is interesting that the turbidity removal was also less with 15 ppm than with 25 ppm of alum. These results indicate that a dose of 15 ppm alum, or about 26 ppm filter alum, approaches the critical dosage for a satisfactory flocculation process under the conditions studied.

The removal of all four organisms in the second stage by 15 ppm FeCl₃ was

not significantly different from that attained by 25 ppm. This relatively improved removal of organisms could be due to the higher turbidity remaining in the supernatant with 15 ppm alum which facilitated a better floc formation with ferric chloride.

To test the above hypothesis a sample of raw Ohio River water having 40 ppm of turbidity was treated with 15 ppm each of both coagulants, but the second-stage flocculation was carried out without removing the floc formed in the first stage. The results showed that removal of the organisms in the first stage was similar to that shown in Table 2, but that removal of the Coxsackie and bacterial viruses was 99 and 99.8 per cent, respectively, in the second stage. These results lend support to the hypothesis and suggest the advantage of using a partially settled supernatant in the second-stage flocculation, particularly if little turbidity is left in the water after the first-stage flocculation.

Although the removal of all four organisms obtained by the two stages combined was consistently higher with 25 ppm than with 15 ppm, the difference

was less significant in the virus than in the bacteria removal. Because the removal of bacteria was relatively poorer with 15 ppm alum in the first stage, it appeared that the two-stage processes in the treatment of raw water with marginal dosages of alum may yield higher removals of viruses than of bacteria. Nevertheless, in spite of this lower removal in the first stage, the total removal was still impressive, i.e., over 99 per cent of viruses and almost 98-99 per cent of the bacteria.

Importance of Contact Time Between Microorganisms and Coagulant Before Floc Formation in the Removal of Organisms in Raw Ohio River Water by Alum Flocculation

In an earlier report⁷ it was shown that efficient virus removal requires participation of the virus in the floc formation. No detectable virus removal was observed when the virus was introduced 30 minutes after dosing the water with 60 ppm of $Al_2(SO_4)_3$. Although this observation disproved the hypothesis that the flocculation process removes particulate organic matter, living or dead, by adsorption of the matter onto

Table 2—Effect of Coagulant Dosage on the Removal of Viruses and Native Bacteria in Raw Ohio River Water by Two-Stage Flocculation at 25 C

Initial Turbidity (ppm)	Stage of Flocculation and Dosage (ppm)	Per cent Removal				Final Turbidity (ppm)	Final pH	Floc Formation
		Coxsackie Virus	Bacterial Virus	Coliform Bacteria	Total Bacteria			
	1st							
60-100	Alum 15 ppm	95.7	85.5	63.8	75.1	5-10	7.1-7.4	Fairly good
16-240*	Alum 25 ppm	98.6	94.8	99.8	99.8	1-5	6.7-7.3	Very good
	2nd							
5-10	FeCl ₃ 15 ppm	94.6	96.5	97.2	90.7	0.1	8.1-8.4	Very good
1-5*	FeCl ₃ 25 ppm	93.8	98.1	93.8	94.8	0.1	7.3-7.8	Good
.....	Combined 15 ppm	99.8	99.5	99.0	97.6	0.1
.....	25 ppm	99.9	99.9	99.99	99.99	0.1

* Reproduced from Table 1 for purpose of comparison.

the formed floc, the importance of the contact time between the organisms and coagulant before floc formation remained to be determined.

In this part of the study an attempt was made, therefore, to define this critical contact time during which the viruses and bacteria must participate in the floc formation if they are to be removed from the treated water. The removal percentages were computed from the results of these experiments, together with pH and turbidity readings, and some observations were made on floc formation (Table 3).

In Table 3 it is seen that 85.0, 76.2, and 75.6 per cent of the Coxsackie virus, bacterial virus, and *E. coli*, respectively, were removed by 25 ppm of $Al_2(SO_4)_3$ when the organisms were introduced

into the water one minute after dosing, whereas when the alum was added to the water containing the organisms, 98.6 per cent of the Coxsackie, 94.8 per cent of the bacterial virus, and 99.8 per cent of the coliform organisms were removed. When the elapsed time between addition of alum and organisms was increased to five minutes or more, none of the organisms were removed. However, when the dosage was increased to 360 ppm, about 60 per cent of the two viruses were removed when they were introduced 30 minutes after the coagulant. The latter result agrees with data observed in a separate study made on purification of viruses by flocculation which will be presented in another report.

The 60 per cent virus removal by 360

Table 3—Effect of Contact Time Between Coagulant and Organism in the Removal of Viruses and *E. coli* in Raw Ohio River Water by Alum Flocculation at 25 C

Dosage of $Al_2(SO_4)_3$ (ppm)	Time Elapsed Between Dosing of Alum and Adding Organisms (min.)	Per cent Removal			Floc Formation, pH and Appearance of Settled Water
		Coxsackie Virus	Bacterial Virus	<i>E. coli</i>	
	0 *	98.6	94.8	99.8 †	
25	1	85.0	76.2	75.6	No floc at time of adding organisms; settled water: pH 6.2-6.4 and turbidity 1-5 ppm
25	5	None	None	None	Floc well formed at time of adding organisms; settled water: pH 6.2-6.4 and turbidity 1-5 ppm
	15	None	None	None	Same as above
	30	None	None	None	Same as above
360	30	62.5	60.3	Thick floc formed; settled water: pH 6.2-6.4 and turbidity 1-5 ppm

* Reproduced from Table 1.
† Coliform bacteria.

ppm of alum suggests that in water dosed with very large amounts of preformed alum floc some aluminum ion may be present to react with organisms in the water. Hence, if preformed alum floc is used, no significant results will be obtained until very large amounts are applied.

It is of interest that the percentage removals of bacterial virus and *E. coli* obtained with 25 ppm alum and an elapsed time of one minute were again slightly lower than that of the Coxsackie virus. These differences could again be explained by the interfering effect of the calcium and magnesium ions in the water, accentuated by the shortened reaction time for the formation of aluminum-organism complexes.

Interfering Effect of Gum Arabic on the Removal of Viruses and Native Bacteria in Raw Ohio River Water by Alum Flocculation

Gummy substances and synthetic detergents are well known for their stabilizing effect on emulsions. Because they form a protective coating on charged particles, one would expect these agents to interfere with the flocculation process. While the interfering effect by the synthetic detergent group has been sum-

marized in the Task Group Report⁸ and further investigated by Smith, Cohen, and Walton of this center,⁹ that of the gummy substances remained to be ascertained.

In a preliminary experiment conducted with 15 ppm of $Al_2(SO_4)_3$ and bacterial virus in raw Ohio River water at 25° C, it was found that the gum exhibited no interfering effect on the removal of the virus until concentrations over 10 ppm were reached. Hence, a limited number of experiments were carried out to determine the interfering effect of 20 ppm of gum arabic on the removal of the Coxsackie virus, bacterial virus, and the native bacteria in raw Ohio River water by 15 ppm of $Al_2(SO_4)_3$ at 25° C. The computed percentages of the removal of all four organisms are shown in Table 4, together with the pH and turbidity readings and observation of floc formation.

As seen in Table 4, the per cent removal of the Coxsackie virus by 15 ppm of alum decreased from 96.7 to 16.7 when 20 ppm gum arabic was added. The percentage removal of other organisms under similar conditions dropped from 93.1 and 94.8 to zero. Although the interfering effect was accompanied by

Table 4—Interfering Effect of Gum Arabic on Removal of Viruses and Native Bacteria in Raw Ohio River Water by Alum Flocculation at 25 C

Nature of Flocculation Process	Per cent Removal				Floc Formation, pH, and Appearance of Settled Water
	Coxsackie Virus	Bacterial Virus	Coliform Bacteria	Total Bacteria	
$Al_2(SO_4)_3$ 15 ppm	96.7	94.8	93.1	93.1	Good floc Formation; turbidity reduced from 15-25 to 0.1-1 ppm; final pH 6.2-6.7
$Al_2(SO_4)_3$ 15 ppm gum arabic 20 ppm	16.7	None	None	None	No apparent floc formation; turbidity slightly increased by the gum; final pH 6.2-6.7

a lack of floc formation, it is believed that the interference is accomplished by preventing the formation of aluminum-organism complexes because the aluminum ion was shown to inactivate bacterial virus,^{3,7} and there was no evidence of such inactivation in the presence of 20 ppm of the gum.

Discussion

At the outset of this discussion, it should be noted that the information obtained with bacterial viruses on the efficiency of a flocculation process has limited application to the removal of pathogenic viruses from water. Being more reactive with bivalent metal ions, the bacterial viruses are removed by flocculation at a higher level in the absence of, and at a lower level in the presence of, such substances as calcium and magnesium ions than are the pathogenic viruses.

The observations made in the present study showed apparently that the removal of Coxsackie virus by alum flocculation in raw river water is slightly lower than that of native bacteria with the difference slightly greater at 5° C–15° C than at 25° C. As a whole, about 95 and 99 per cent of the Coxsackie virus and about 99 and 99.8 per cent of the native bacteria are removed by 25 ppm of alum, i.e., 44 ppm or 2.5 gpg (grains per gallon) of filter alum at 5° C–15° C and 25° C, respectively, in raw river water having turbidity levels under 260 ppm.

The significantly lower removal of the bacteria and bacterial virus and fair floc formation obtained with 15 ppm of alum (26 ppm or 1.5 gpg of filter alum) indicate that this dosage was at or close to the critical level. The fact that the residual turbidity was quite low at both dosage levels implies that coagulant doses adequate for turbidity removal are not necessarily high enough to insure efficient virus or bacterial removal.

The organism removals obtained in the second stage using FeCl_3 cannot be applied directly in estimating the removal of viruses and bacteria in a raw water because the supernatant obtained from the first stage in this study, and used in the second stage, was relatively free of turbidity with considerably higher calcium ion content and pH value. Even under these conditions, 15–25 ppm or about 0.9–1.4 gpg of FeCl_3 facilitated about 95 per cent removal of the Coxsackie virus. The lack of improvement in removal efficiency when the dosage of FeCl_3 was increased from 15 to 25 ppm was probably due to insufficient suspended material left in the supernatant to promote good floc formation.

From the results obtained with the two stages of flocculation combined, one sees that the removal of the Coxsackie virus in the raw water by 25 ppm of both alum and FeCl_3 was 99.6, 99.6, and 99.9 per cent at 5° C, 15° C, and 25° C, respectively. On the other hand, the native bacteria removal was 98–99 and 99.99 per cent with 15 and 25 ppm dosages, respectively, at 25° C, and about 99.9 per cent with 25 ppm at 5° C and 15° C.

The observation regarding the critical contact time for forming organism-coagulant complex has both theoretical and practical implications. In theory it confirms the previous observation⁸ that the removal of viruses in water by this process involves, first, a reaction consisting of coagulant-cation-virus complex formation and, second, the formation of aggregates of these complexes, and that the first reaction is completed in a short time. In practice, it emphasizes the importance of rapid and complete dispersion of the coagulant chemicals in the water to be treated and the insignificant value of preformed floc in removing microorganisms.

Demonstration of the interfering effect of gum arabic on the removal of viruses

and bacteria in water by alum flocculation probably has more theoretical than practical interest. It illustrates again the importance of the formation of the coagulant-cation-organism complex as the first step in the removal mechanism and the likelihood of low removal efficiencies when interfering substances are present.

Recent published reports combine prechlorination and flocculation when comparing the relative efficiencies of stages in the removal of bacterial organisms in water treatment and no information is available regarding the comparative efficiencies of virus removals. Streeter's reports on the survey of filtration plants along the Ohio River in 1923-1924¹⁰ and an experimental study made with Ohio River water in 1926¹¹ showed that the bacterial removal efficiency of alum flocculation as practiced at that time was decidedly lower than that attained in the present study. Streeter's reports also showed higher levels of residual turbidity in the treated water. Since it was common practice in those days to carry out the flocculation process in such manner as to leave some turbidity in the settled water in order to improve the removal efficiency of the filtration process, the bacteria removal of the flocculation process was naturally lower than that obtained in the present study. A fair comparison, therefore, cannot be made.

Improvements in flocculation processes have enabled modern waterworks operators to apply these procedures in the field with maximum practical efficiency in removing suspended matter. To insure this efficiency the information gathered in the present studies indicates that the following points should be observed:

1. The dosage of coagulant should be adequate to insure good floc formation.
2. If the raw water carries synthetic detergents and/or similar substances at levels that would interfere with the

process, the dosage of coagulant should be appropriately increased to counteract the interference and promote good floc formation.

3. The pH regulation of the raw water should be such that it will not cause a floc formation sufficiently rapid to reduce the contact between the coagulant cation and the suspended particles during the period of floc formation, nor too slow a floc formation to interfere with the settling of the formed floc.

4. The stirring rate employed during the flocculation period should be fast enough during the first few minutes to provide a good contact between the flocculant cation and the suspended particles and should be adjusted after this critical period to facilitate the formation of floc masses that will settle out within the prescribed period.

5. Because of the apparently interfering effect of calcium and magnesium ions on bacterial removal, it appears that flocculation of water having unusually high contents of these ions would require preflocculation treatment to reduce the metal ions or a corresponding increase of coagulant dosage, especially when the process is applied to cold water. The use of lime to raise the pH of the water should be replaced by the use of caustic soda or compounds containing no calcium or magnesium.

6. In a two-stage process the removal efficiency of the second stage may be improved by incomplete settling of the floc in the first stage, which leaves enough suspended material to form a good floc in the second stage.

Summary and Conclusions

A study has been conducted to determine (1) the efficiency of a two-stage flocculation process for removing Coxsackie and bacterial viruses and native coliform and total bacteria in raw Ohio River water at 5° C, 15° C, and 25° C, (2) the critical time in relation to floc

formation for the removal of these viruses and *E. coli* in the flocculation period, and (3) the interfering effect of gum arabic on the removal of these viruses and native bacteria in raw river water by alum flocculation. From the observations made in this study, the following summary is prepared:

1. Based on the results obtained in the first stage, removal of the Coxsackie virus by 25 ppm of alum, i.e., 44 ppm of filter alum, in raw river water having turbidity readings under 260 ppm was 95 and 99 per cent at 5° C–15° C and 25° C, respectively, and by 15 ppm of alum, i.e., 26 ppm of filter alum, 95.7 per cent at 25° C. In the same samples removal of the bacteria showed insignificant differences between *E. coli* and total bacteria. It was slightly higher than Coxsackie virus removal with 25 ppm and significantly lower with 15 ppm of alum, with removal of the bacterial virus slightly but consistently lower than that of the Coxsackie virus.

2. The virus-removal efficiency of the FeCl_3 used in the second stage was comparable to that of alum, but its bacterial-removal efficiency was lower. These relatively poor results were attributed to poor floc formation due to inadequate turbidity. The lower bacterial removal was enhanced by the interfering effect of about 100 ppm of calcium ion and over 10 ppm of magnesium ion, the effect of which was exaggerated at lower temperatures.

3. Removal of Coxsackie virus by the two stages combined was 99.6 per cent at 5° C and 15° C, and 99.9 per cent at 25° C with 25 ppm coagulant in each stage. With 15 ppm coagulant, Coxsackie removal was 99.8 per cent at 25° C. At the same time removal of native bacteria was 98–99 and 99.99 per cent by 15 and 25 ppm dosages, respectively, at 25° C, and about 99.99 per cent by the 25 ppm dosage at 5° C and 15° C.

4. Removal of the Coxsackie virus

was slightly higher in general than that of the bacterial virus, contrary to the findings in the preceding study¹ made with water of known chemical content. The presence of calcium and magnesium ions in the raw water and the addition of CaO in the second stage were believed to have interfered with the formation of the coagulant-cation bacterial-virus complex.

5. The critical time for the formation of coagulant-microorganism complex in relation to removal of viruses and *E. coli* by practical dosages of alum at 25° C was confined to the first few minutes after addition of the coagulant. The removal of about 60 per cent of the Coxsackie and bacterial virus by 360 ppm of alum when the viruses were introduced 30 minutes after the alum addition was attributed to the presence of a small amount of aluminum ion in combination with this large coagulant dose.

6. Gum arabic at a concentration of 20 ppm was found to prevent completely the formation of floc and removal of Coxsackie and bacterial viruses and native bacteria in raw Ohio River water dosed with 15 ppm of alum.

As a result of these observations, a number of suggestions were presented for obtaining practical maximum efficiency in removing viruses as well as bacteria and other particulate matter in water supplies by flocculation.

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Geriatric Rehabilitation Course

Principles and Practice of Geriatric Rehabilitation is a two-week course to be given by the New York Medical College-Metropolitan Hospital Center, April 21 to May 2. Designed for registered nurses, occupational and physical therapists, and social workers, the course is planned to provide intensified training in the rehabilitation care of the elderly, chronically ill patient.

Tuition for the course is \$100. A limited number of traineeships may be available through a grant from the U. S. Office of Vocational Rehabilitation. New York State scholarships are also available to state residents or to those who expect to work in the state. Request for scholarships should be made at the time of application which should be made as early as possible since enrollment is limited. Dr. Jerome S. Tobis, Director, Department of Physical Medicine and Rehabilitation, New York Medical College, One East 105th St., New York 29, N. Y.