1-1-1974

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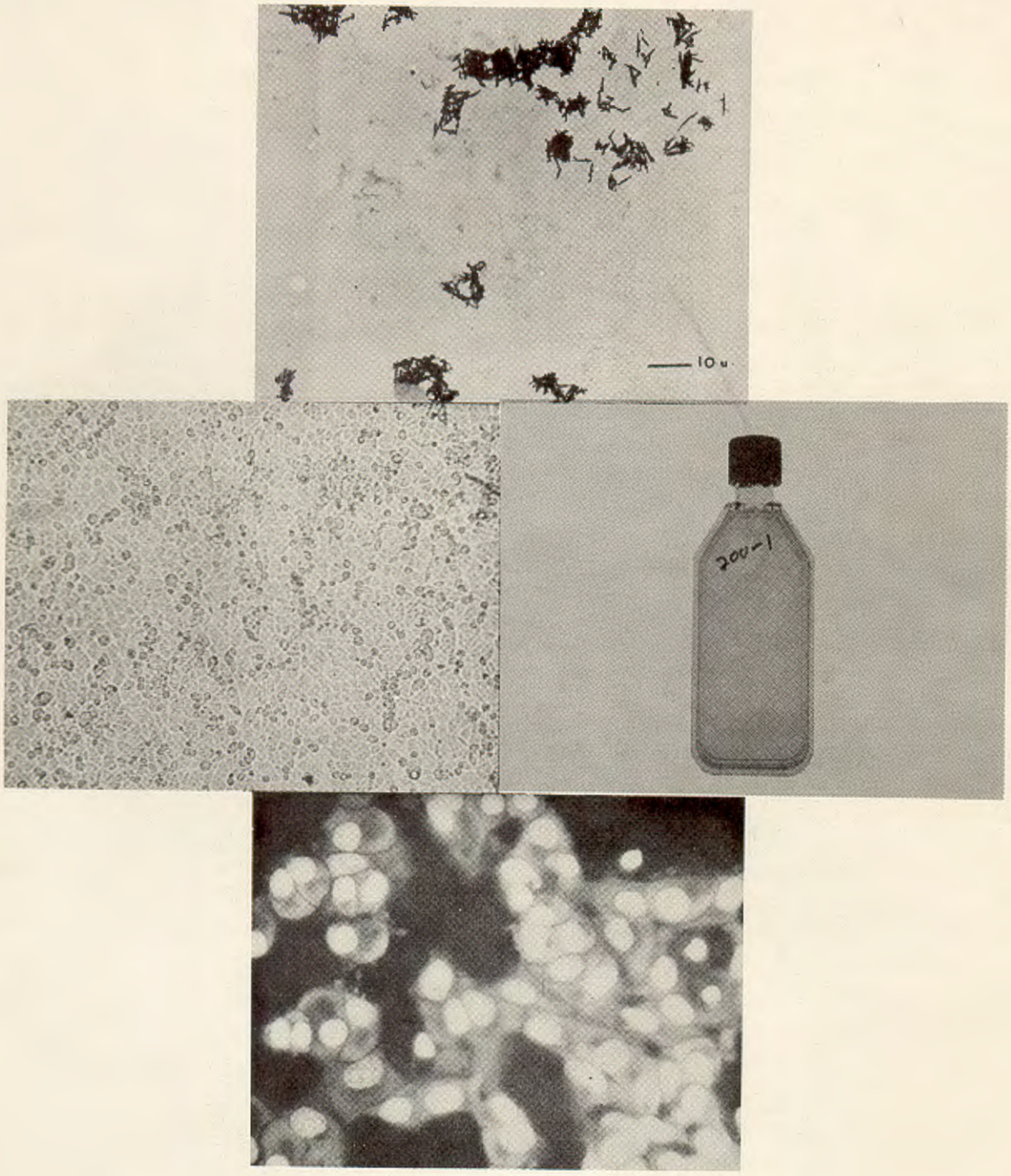

Research Report No. 10

WATER RESOURCE RESEARCH CENTER
UNIVERSITY OF NEW HAMPSHIRE
DURHAM, NEW HAMPSHIRE

THE USE OF MAGNETIC IRON OXIDE FOR RECOVERY OF VIRUS FROM WATER

by

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COMPLETION REPORT

Project No. A-014-NH

under Grant #14-31-0001-3029

The work upon which this publication is based was supported in part by funds provided by the Office of Water Resources Research, United States Department of the Interior, as authorized under the Water Resources Research Act of 1964, Public Law 88-379.

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ABSTRACT

Magnetic iron oxide was found to consist of a mixture of the gamma spinel of Fe_2O_3 and alpha spinel of Fe_3O_4. Electron micrographs showed a long, irregular, rod-like structure. Virus interaction with magnetic iron oxide was believed to occur at multiple combining points present at each octahedral site of an Fe atom. The interaction was considered to be an adsorptive process. The degree of adsorption was dependent upon the electrostatic potential in effect between iron oxide and virus coat protein surfaces.

The potential developed was a function of structural protein ionization, and its extent the result of solution pH relative to protein isoelectric point. Virus interaction with magnetic iron oxide was independent of iron oxide arrangement in columns or thin sandwich-like layers. Virus recovery was most effective when elutions from thin layers were made. Adsorption was favored by acid pH while elution was best at alkaline pH. Virus recoveries of 90 percent were made from litre samples of distilled water. Experimental findings led to a prediction of a limited role for use of magnetic iron oxide for recovery of virus from surface waters.

INTRODUCTION

Demonstration of an enterovirus in surface waters presumes an ability to detect the presence of a few virus particles in large volumes of water. One of the early and more successful procedures used by virologists for detecting the presence of virus in surface waters was the gauze pad method originally introduced by Moore (1948) for detection of paratyphoid carriers. MacCallum (1952) reported more virus isolations were obtained from sewage when gauze pads rather than "grab" samples were used. Kelly (1953) and Melnick et al (1954) adapted this procedure to the recovery of Coxsackie-virus from sewage. Later, Kelly et al (1955) showed the gauze pad method to be effective for isolation of enteroviruses occurring intermittently in sewage. Conversion of a qualitative to a quantitative procedure was claimed by Coin et al (1964), who designed and introduced a flow-through gauze sampler which was used successfully for isolation of enteroviruses from finished and raw waters in France. The effectiveness of the flow-through gauze sampler for enumeration of virus in water was found to be low. Effectiveness furthermore was found to depend upon the presence of a virus adsorption enhancing effect (Liu, et al, 1971). Clay particulates and salt concentration contributed to enhancement of virus adsorption. Ready adsorption of virus to a number of adsorbent materials including resins has been demonstrated (Schwerdt, 1965). Ion exchange resins used successfully for partial purification of poliovirus include amberlite (Lo Grippo and Berger, 1952) and Dowex resins (Taylor and Graham, 1958).

The possibility of a new approach to the problem of enterovirus detection and enumeration in water was suggested by the outcome of an accidental laboratory finding. Warren et al, (1966) discovered addition of magnetic

iron oxide to a myxovirus suspension led to removal of 90 percent of its virus content. Rao et al, (1968) were prompted to test the ability of magnetic iron oxide to remove enterovirus from water. Recoveries of 87 to 100 percent of initial virus numbers were obtained upon elution of columns of magnetic iron oxide following passage of virus samples under positive pressure.

The following study describes the development of equipment and methods which sought to combine the virtues of flow-through procedures with the virus-removal capabilities of magnetic iron oxide. Factors influencing virus-iron oxide interaction and the effectiveness of equipment and methods for recovery of virus were examined. Special attention was directed to theoretical aspects of virus-iron oxide interactions, and an explanation of the basis of interaction sought.

MATERIALS AND METHODS

Virus sampling equipment

The first sampler was designed and fabricated at the University of New Hampshire. It consisted of a March model MOX-35 magnetic drive, fan-cooled pump which forced water being sampled for virus through two collecting columns arranged in series. Each column was 2 inches in diameter and 18 inches long. Each was one third full of magnetic iron oxide. Rubber stoppers fitted with glass tubing prevented sample loss during column operation. Tygon tubing connected columns with each other and with pump and flowmeter as well. The pump was energized by two six volt batteries, with an ATR DC-AC inverter[1] inserted between batteries and pump. Batteries and transformer made it possible to use the sampler in the field for "remote site" sampling. Following passage of water through the iron oxide columns, it was discharged through the flowmeter where sample volume tested was determined. Virus was recovered from iron oxide columns by application of an eluting fluid. The sampler is shown in Plate I. A second sampler suitable only for laboratory trials consisted of a 142mm Millipore membrane filter holder with magnetic iron oxide arranged in a thin sandwich-like layer. The layer was maintained by means of AP20 filters positioned above and beneath the iron oxide. Samples contained in a pressure cylinder were passed through the iron oxide under positive pressure supplied by a nitrogen cylinder. A baffle inserted in the influent port of the filter holder distributed the water flow evenly over the entire iron oxide surface and prevented channeling. The equipment is shown in Plate 2.

[1]ATR Manufacturing Company, St. Paul, Minnesota

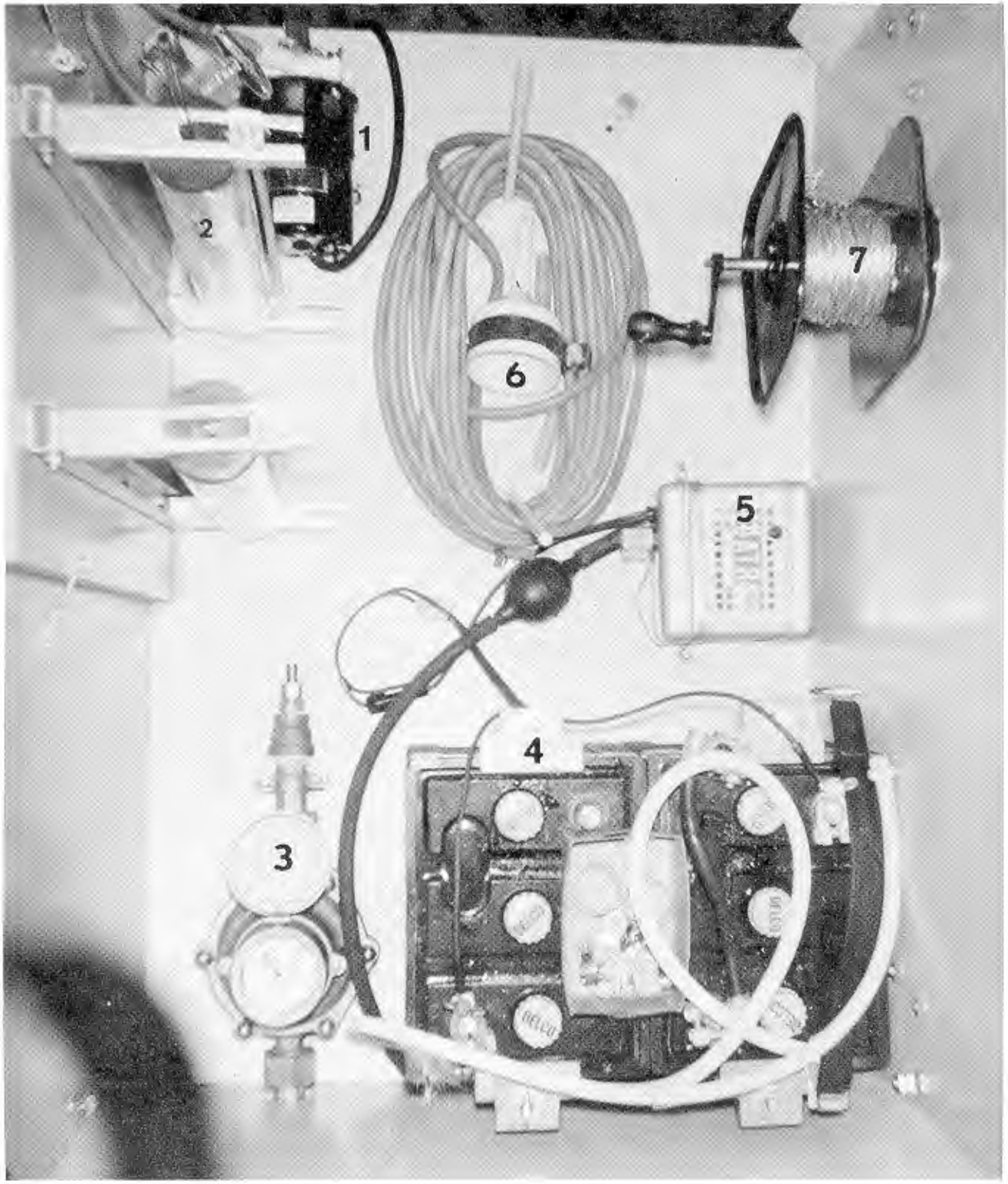

Plate 1. Remote station Flow-Through Virus Sampler. (1) pump (2)virus adsorbent columns (3) Flow meter (4) Batteries (5) DC-AC inverter (6) Sample intake hose and funnel (7) crank and wire for lowering intake hose in water.

Plate 2. Laboratory Flow-Through Virus Sampler

(1) Nitrogen chlinder (2) Pressure regulating gauges (3) Pressure vessel (4) 142mm filter holder with magnetic iron oxide (5) Sample discard container

Magnetic iron oxide

The iron oxide was obtained from Charles Pfizer Company in the form of an amorphous powder, MO 2530. To prepare it for use, 100gm portions were washed 3 times with distilled water to remove small floating particles. The wet slurry at the bottom of the wash container was dried at 100°C for 20 hours and used in this form. If sterile conditions were required, the dried powder was sterilized in 25gm amounts for 1 hour at 150°C.

Twenty-five gm of magnetic iron oxide was added to 400ml deionized water and shaken manually. After a 16 hour hydration period the oxide was layered on an AP20 filter pad with the help of negative pressure. All filter pads were treated with a 0.1 percent tween 80 solution prior to use.

X-ray diffraction analysis was made using powder pattern technique and high resolution x-ray powder diffraction camera equipment.[1] Magnetic iron oxide in 0.582gm amounts was placed in a glass capillary tube with 0.3mm internal diameter and a wall thickness of 0.01mm. The crystallography was studied by consideration of 12 d values for Fe_2O_3, and 8 d values for Fe_3O_4. Radiation exposure of FeK_X was 1 hour at 35 KV. Ilford type G film was used.

Electron microscope examination was made using a Philips EM-200 instrument. Dilutions providing adequate material for examination were prepared in double distilled water. The dilutions were placed on formvar coated 300 mesh copper grids[2] and examined directly without staining.

Virus collection

Samples were adjusted to pH 5.0 by addition of 0.1N HCl and passed through magnetic iron oxide at flow rates of 1 to 2 lpm. Filtrates were discarded after removal of test samples for virus assay. Virus was recovered

1/ 57.3mm diameter Debyre-Scherrer cylindrical model, Siemens and Halske AG, Karlsruhe, Germany.
2/ #2214, Ernest F. Fullam, Inc.

from oxide layers by elution using either 3 percent isotonic beef extract solution[1], pH 9.0, or Eagles minimal essential medium containing 10 percent fetal calf serum. An eluent volume of 250ml was found most effective for recovery of virus from iron oxide layers.

Virus assays

Assays were made using as an end point the dose infective for one-half of the cell culture monolayers inoculated ($TCID_{50}$ dose). Assays were conducted using African Green primary monkey kidney [2] or the $LLCMK_2$ established cell line (Hambling and Davis, 1965). Test tube monolayers with 1.0 ml liquid overlay received 0.1 ml inocula. Six, eight or ten monolayers were used for each dilution tested. Tests were read for cytopathic effects (CPE) at daily intervals for a total of 10 days.

Enteroviruses used

Poliovirus I (chat), Coxsackievirus B3 (Nancy), and Echovirus 6 (D'Amori), obtained from the American Type Culture Collection and maintained within the Virus Laboratory, Department of Microbiology, were used during the studies. Virus stocks propagated on $LLCMK_2$ monolayers were maintained at -70°C. Stock suspensions possessed titers of $1x10^6$ $TCID_{50}$ or better per 0.1 ml.

1/ Beef Extract obtained in paste (Difco) or powder (Wilson Diagnostics, Inc.) form.

2/ Flow Laboratories, Rockville, Maryland

RESULTS

Removal of virus by magnetic iron oxide was studied using the remote site sampler and membrane filter equipment. Virus suspensions were pumped through iron oxide columns in the remote site sampler. Positive pressure supplied by nitrogen was used to force virus suspensions in pressure cylinders through thin layers of iron oxide within the membrane filter holder. Filtrates were assayed for virus. The results are given in Table I.

Virus penetration of magnetic iron oxide occurred under both of the sampling conditions imposed. No significant differences between the two experimental systems were found. The results indicated virus removal was similar in each system, and differences in iron oxide arrangement did not lead to differences in virus removal effectiveness. Magnetic iron oxide was equally effective in removal of virus whether used in columns or thin layers. This indicated the brief contact between virus and iron oxide experienced with thin layers served the same purpose as the more prolonged contact in columns. The results suggested a strong attraction with immediate interaction potential existed between virus and magnetic iron oxide.

Elution of virus from magnetic iron oxide was examined for both of the experimental systems. Two eluents, a 3 percent beef extract solution and Eagles MEM with 10 percent fetal calf serum were compared. The eluents were passed slowly through magnetic iron oxide in 250 ml volumes. Eluates were collected and tested for virus. The results of a series of trials are given in Tables 2 and 3.

Greater virus recoveries were obtained from thin layers than columns of magnetic iron oxide. The greater recoveries obtained from thin layers resulted from more effective elution rather than more effective removal of

virus. No significant difference in virus removal by either layers or columns of iron oxide was shown. The greater recoveries made with beef extract indicated it was superior to Eagles MEM for elution of virus.

The improved virus recoveries obtained using thin layers of magnetic iron oxide were accomplished with one-thirteenth the amount of iron oxide present in columns. The lack of significant removal differences indicated only that test suspensions of 1000 ml did not exhaust the removal capability of the smaller amount of magnetic iron oxide present in thin layer form. The improved effectiveness of elution was subject to at least two interpretations. Distribution of magnetic iron oxide in thin layers promoted more effective contact between virus and eluent, resulting in greater release of virus. The greater amount of iron oxide in columns required an eluent volume in excess of the 250 ml used. Since both interpretations really suggested thin layers of iron oxide to be more efficient for elution, it was decided to determine how much magnetic iron oxide really was needed for virus removal under idealized conditions.

Constant amounts of virus in unit volume were added to increasing amounts of iron oxide suspended in 10 ml of phosphate buffered saline, pH 5.0. Contact between virus and iron oxide was promoted by mild shaking for 30 minutes, after which the tubes remained stationary for another 30 minutes. Virus removal was measured by testing supernates for free virus. The results are shown in figure I.

The rate of virus removal by magnetic iron oxide increased rapidly. Fifty percent removal occurred with 5 mg/ml. More than 75 percent of virus had been removed from iron oxide supernates with 10 mg/ml. Supernates were virtually free of virus at 15 mg/ml and completely free at 20 mg/ml. No reversal of removal occurred at concentrations of iron oxide up to 40 mg/ml.

Rao et al (1968) reported virus elution from magnetic iron oxide to be best at pH 8. The previous experiences of several investigators who reported good elution of virus from gauze pads at a higher pH prompted an examination of the effect of higher pH upon virus elution from magnetic iron oxide. Ten milliliters of a virus suspension possessing a titer of $5X10^6$ $TCID_{50}$/ml was passed through 0.25 gm of iron oxide at pH 6 in a series of test tubes. The supernatant fluids were removed and replaced with equal volumes of 3 percent beef extract solution having final pH values of from 7 to 10.5. The test tubes were shaken gently for 10 minutes, the iron oxide allowed to settle and supernates tested for virus. The results are shown in figure 2.

The influence of pH upon elution was decisive. Elution effectiveness increased rapidly from pH 7 to the vicinity of pH 9.5. More than 90 percent of virus was recovered at pH values of 9 to 10. Recoveries were poor at pH values of less than 9, and there was indication of a decline at values greater than 10.5.

Recovery of enterovirus from water was studied next. One liter distilled water suspensions of virus at pH 6 were passed through thin layers of 25 gm magnetic iron oxide under positive pressure. Elution of virus was carried out with 250 ml of isotonic 3 percent beef extract, pH 9.0 (powdered, Wilson Diagnostics), passed slowly through the iron oxide. Virus recovery was evaluated on the basis of the percent of initial virus found in eluates. The results are given in Table 4.

Recoveries of 79 to 93 percent were obtained with a 3 percent beef extract eluent. Virus was found in only two filtrates, and in small amounts in each instance. Not all of the virus removed by magnetic iron oxide could be recovered, but the fraction not eluted was small and elution effectiveness was considered good. Similar recoveries for each of 4 enteroviruses indicated

no drastic difference in elution effectiveness need be anticipated a priori for members of different groups of enteroviruses.

Interference by organic substances with virus recovery from water has been described previously (Wallis and Melnick, 1967). The recoveries achieved with beef extract suggested use of magnetic iron oxide along with the components of beef extract as elements of a model system for the investigation of iron oxide-virus interaction. Such a system would offer twin advantages of simplicity and use of simple organic structures. It was reasoned that information on the nature of iron oxide-virus interaction could be obtained from a consideration of the interaction interference contributed by beef extract components.

The availability of the individual components found in beef extract in isolated form provided an opportunity to test this hypothesis. Three percent solutions of several components at pH 6.0 were passed through individual iron oxide layers in separate trials, followed by distilled water rinses. Virus solutions of known assay values and adjusted to pH 6 were then passed through the treated iron oxide and filtrates examined for virus. The degree of inhibitory effect shown by a component was measured by the amount of virus appeating in a filtrate. The results of a series of trials are shown in Table 5.

Proteose peptone, creatinine, basic amino acids and vitamins provided little interference with virus recovery. Peptone and high pKa amino acids, however, were effective inhibitors and approached closely the degree of interference displayed by beef extract. The results suggested high pKa amino acids which are common to both peptone and beef extract were responsible for the interference. The data pointed toward the importance of positively charged points on iron oxide for virus-iron oxide interaction. Amino acid ionization conferring a net negative charge from

carbonyl group participation at pH values on the acid side of amino acid isoelectric points would lead to development of electrostatic forces attracting negatively charged structures to positively charged surfaces.

The results of the interference tests led to further examinations in which high pKa amino acids along with organic substances having functional amino nitrogen groups were screened for their virus elution properties. The data is given in Table 6.

High pKa amino acids and Earles balanced salt solution with 2 percent fetal calf serum were both highly effective in their elution of virus from magnetic iron oxide. Uracil and xanthine were poor eluents and sperm ine and glucosamine were almost without effect. Glucose and streptomycin were inactive. The results indicated electrostatic forces similar or identical to those postulated responsible for inhibition of virus interaction with iron oxide were also responsible for elution of virus. The results indicated the same or closely associated sites on magnetic iron oxide were involved in both virus interaction and virus elution phenomena.

Consideration of the ionization characteristics at pH 5 and 9 of four amino acids known to occur in poliovirus coat protein illustrates a theoretical concept of changes occurring in surface charge during virus-iron oxide interactions (Levintow and Darnell, 1960). A related consideration of amino acid functional groups involved in hydrogen bonding provides further insight into a relationship between pH, surface charge and bonding influences. Figure 3 and Table 7 illustrate functional groups involved.

Cysteine and histidine would exhibit a net negative charge at pH 9, while lysine and arginine would show a lowered degree of positive charge. Magnetic iron oxide could be considered as an acceptor at OH^- or OH dominated sites for NH_3^+ or sulfhydryl groups of virus coat protein at

pH 5. Elevation of pH to 9 for elution causes NH_3^+ to become an amino group, and sulfhydryl a sulfide radical, with corresponding changes in reactivity.

X-ray diffraction patterns, emission spectra and electron micrographs were studied to determine the type, structure and purity of magnetic iron oxide. The diffraction pattern is shown in Table 8 and other elements in iron oxide in Table 9. The x-ray pattern is given in Plate 3 and electron micrograph in Plate 4.

The data compiled from these analyses indicated magnetic iron oxide was a gamma spinel of Fe_2O_3 with traces of Fe_3O_4. The unit cell of the spinel was composed of 22 iron atoms distributed randomly among 8 tetrahedra and 16 octahedra. A total of 32 oxygen atoms occurred in the basic unit. Other metals, especially cobalt, manganese, nickel, zinc and molybdenum could substitute randomly in the lattice to replace a small number of iron atoms. The gamma spinel (Fe_2O_3) had an average of 21 1/3 Fe atoms, compared to the usual 24 for the alpha form (Fe_3O_4). The crystal structure postulated for magnetic iron oxide is shown in Figure 4.

Electron micrographs of magnetic iron oxide show a long, rod-like structure. The aggregation seen in the micrographs was not observed macroscopically in hydrated suspensions. In aqueous solutions the spinel form would break down and hydrated iron complexes would result. Multiple combining sites for potential interaction with virus would occur. With the 4 combining sites per octahedral site of an Fe atom shown in Figure 4, the 22 to 24 Fe atoms per spinel unit would provide a theoretical possibility of as many as 96 potential virus receptor sites.

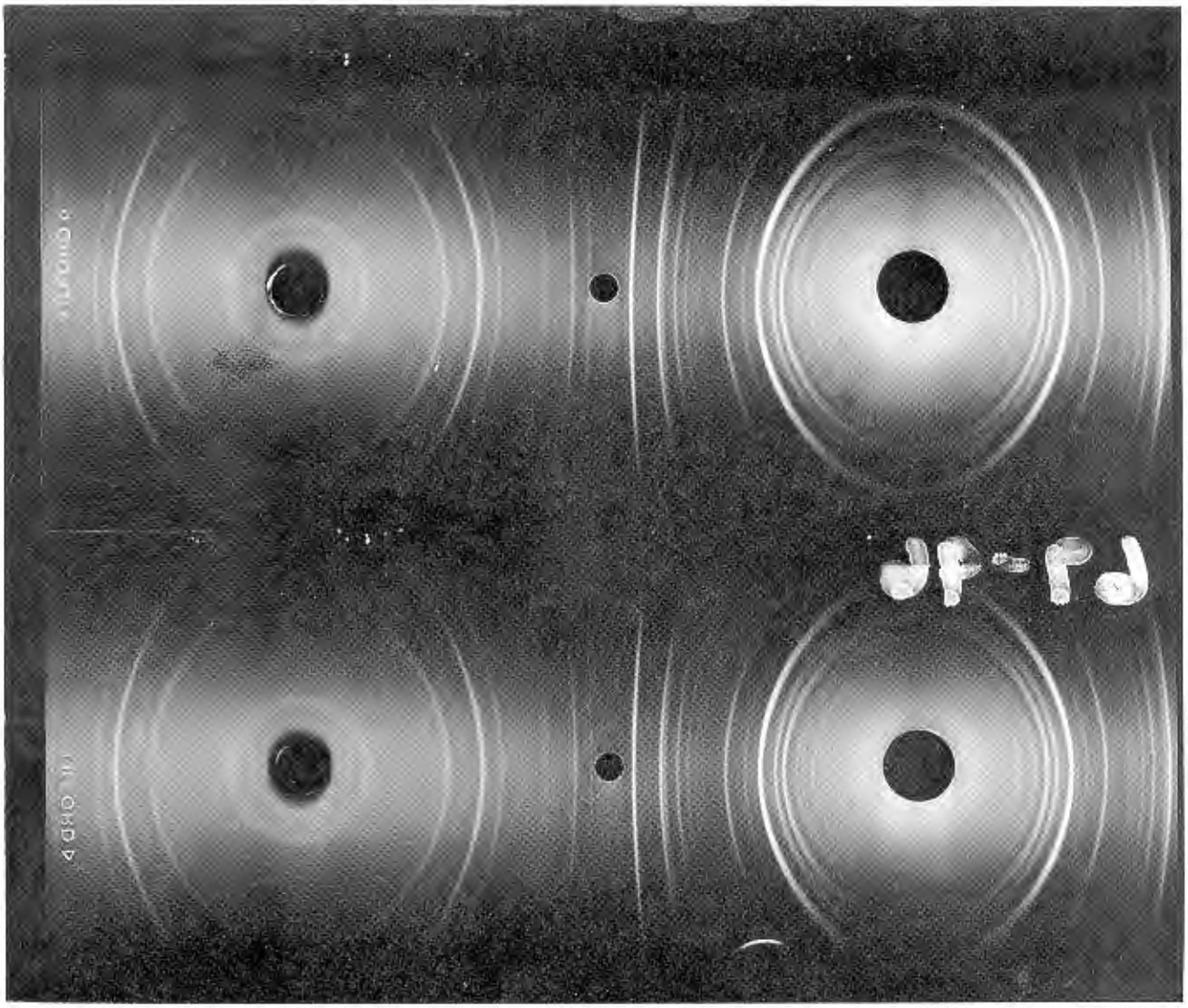

Plate 3. X-Ray Diffraction Powder Pattern of Magnetic Iron Oxide

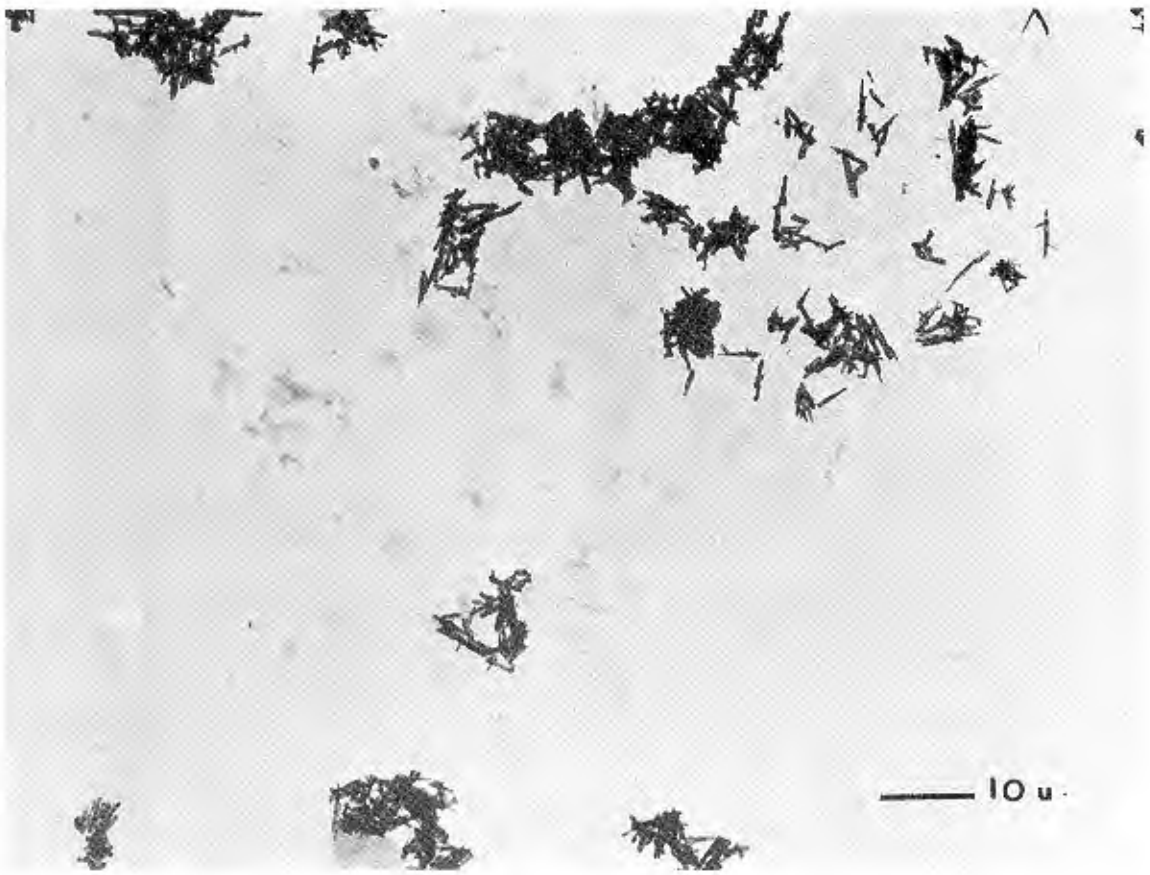

Plate 4. Electron Micrograph of Magnetic Iron Oxide.

DISCUSSION

Recovery of virus from aqueous solutions by magnetic iron oxide was considered to be the result of a physical adsorptive process. The process was dependent upon the electrostatic potential in effect between virus and iron oxide surfaces. Any influence increasing this potential let to increased adsorption. Elution of virus was possible only after significant decrease of potential was made. The electrostatic potential developed using magnetic iron oxide was conceptualized in terms of oxide atomic structure in hydrated form. A mixture of gamma and alpha spinels of iron oxide provided 4 combining sites for each of 22 or 24 iron atoms per spinel unit. A possible availability of as many as 96 virus combining sites per unit illustrated at least one reason for the virus adsorbing capabilities shown by magnetic iron oxide. Each combining site was considered a basis for the combination demonstrated with amino acid structures of ionized protein surfaces. The degree of electrostatic potential developed not only was a function of ionized amino acids but depended upon virus solution pH relative to the isoelectric point of the virus protein involved.

A theoretical basis for changes in the electrostatic potential between virus and adsorbing surface which conforms to study models was provided by Levintow and Darnell (1960). Only 25 percent of the amino acid content of poliovirus coat protein formed from 16 amino acids showed significant changes in charge at pH values 5 and 9. Cysteine became negative ($SH \rightarrow 5$), histidine became less positive ($N^+ \rightarrow N$), and distal amino acid groups of arginine and lysine became less positive ($NH_3^+ \rightarrow NH_2$). Study data indicated charge density changes of this sort to be important in enhancing or

depleting the degree of electrostatic potential in effect. PH adjustment of a virus solution to 5 was considered to increase adsorption by increasing the potential gradient. Addition of salt further enhanced the charge differential. PH adjustment of the same solution to 9 decreased or reversed the sign of the gradient. The result would be a diminution of attraction or increase in forces leading to repulsion of virus from iron oxide surface.

Alternative explanations considered for virus binding by magnetic iron oxide included covalent bond formation, hydrogen bonding and long range Van der Waals forces. The possibility of covalent bond formation was discarded on the basis of the energy required to break the bonding association between virus and oxide. A calculated value of 35 KCal/mol was estimated to be necessary. No mechanism for an input of energy of this magnitude existed. Hydrogen bonding between hydrated oxide and epsilon amino group of lysine, the terminal amino group of arginine or histidine was possible. Sulfhydryl, phenolic, imidazole or quanidinium structures were addition possibilities. The prospects for hydrogen bonding could not be evaluated experimentally due to the toxicity for cell cultures of bond-breakers like urea and triton 100X. Van der Waals forces would seem unlikely in view of the surface area necessary to insure the degree of bonding firmness found during the study. The total surface area presented by virus in concentrations considerably greater than those encountered in the study theoretically would still prove inadequate for such bonding firmness.

Magnetic iron oxide was more effective for recovery of virus when arranged in thin layers than in column form. The greater efficiency of elution from thin layers rather than initial interaction was responsible for this effect. The greater elution efficiency was attributed primarily

to a more effective contact between eluent and adsorptive surface. Secondary iron oxide contact of released virus occurring in columns would not be expected in thin layers.

The experimental findings predicted a limited role for use of magnetic iron oxide for recovery of virus from water. Evidence of the adsorptive nature of virus-iron oxide interaction meant a variety of structures could be expected to interfere with virus recovery. Adsorption of colloidally dispersed particulate matter could be expected to clog iron oxide layers, preventing free passage of sample and restricting water volume possible to examine. Organic matter could be expected to interfere by selective binding at virus adsorbing sites. Structures possessing greater binding potential than virus would be bound preferentially, denying virus access to these bonding points.

ACKNOWLEDGEMENTS

The assistance of Wayne Beasley, Associate Professor of Mechanical Engineering, in the preparation and interpretation of X-ray diffraction patterns is gratefully acknowledged.

The help of Emory Clippert, Assistant Electron Microscopist, in the operation of the Philips EM-200 electron microscope is acknowledged with appreciation.

The design and assembly of the remote site flow through virus concentrator by the Center for Industrial and Institutional Development personnel is acknowledged.

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FIGURE I

The Effect of Magnetic Iron Oxide Concentration Upon Virus Removal

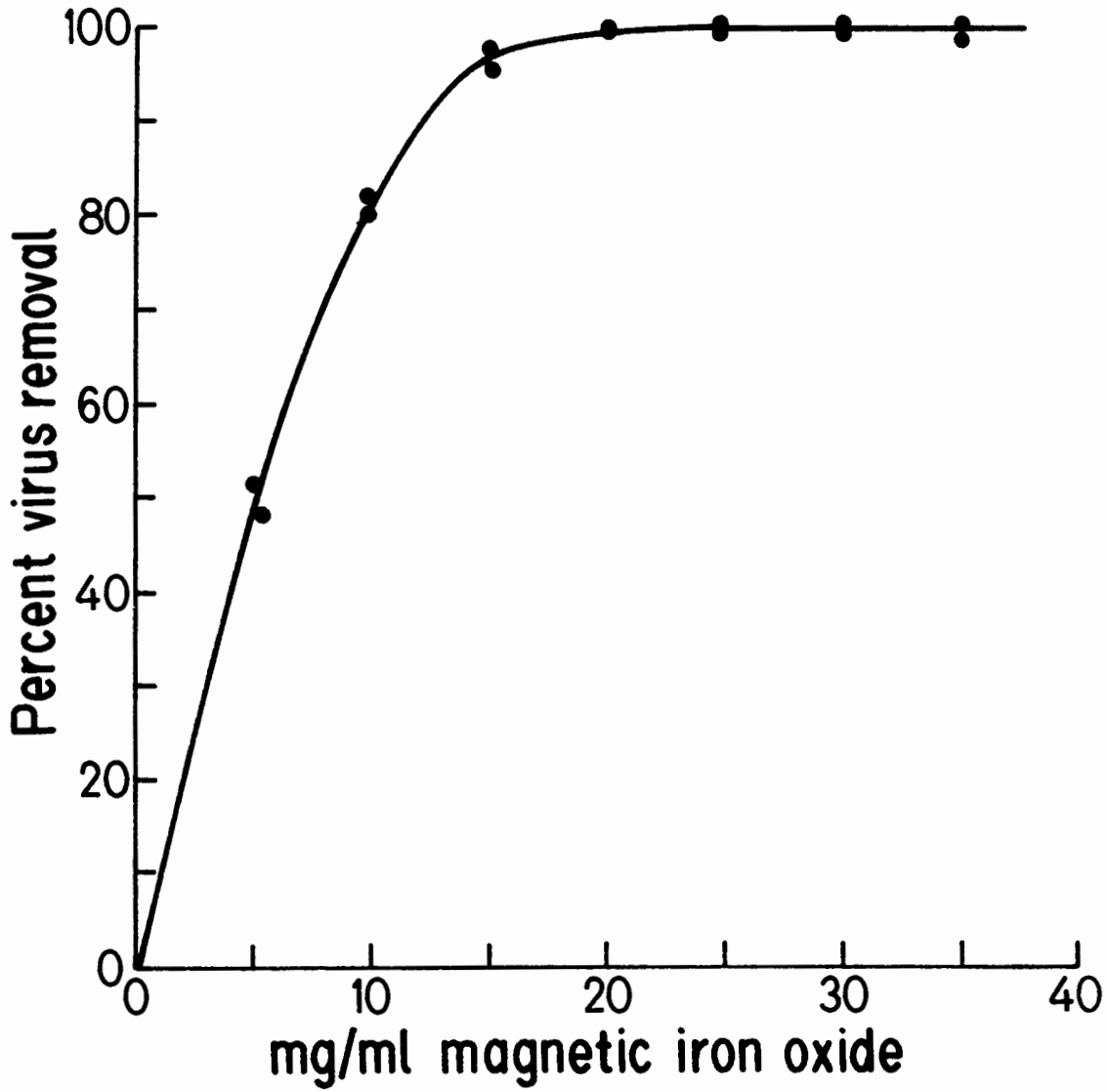
Percent virus removal
100
80
60
40
20
0
0
10
20
30
40
mg/ml magnetic iron oxide

FIGURE 2

The Effect of pH Upon Virus Elution from Magnetic Iron Oxide

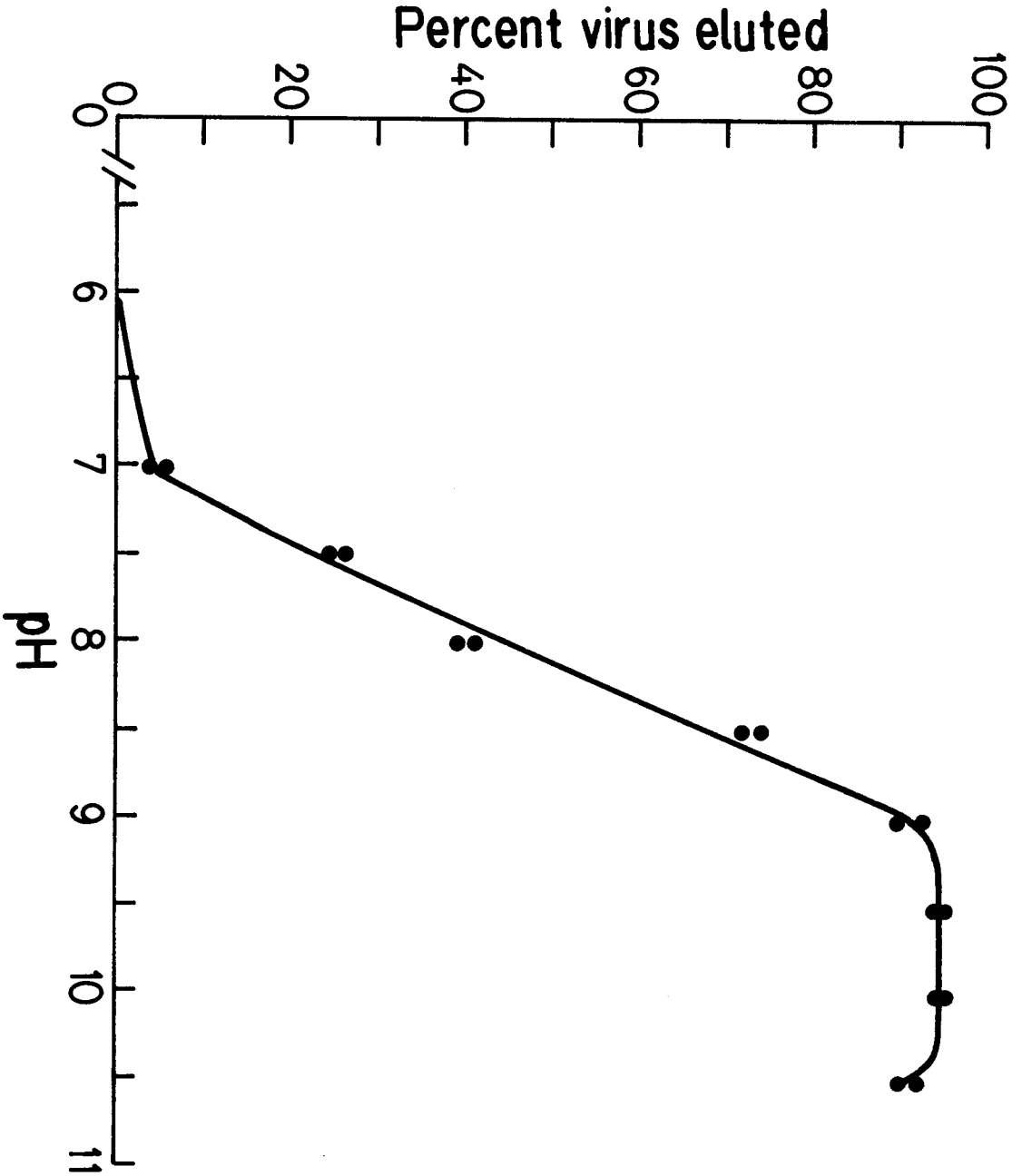
Percent virus eluted
0
20
40
60
80
100
pH
6
7
8
9
10
11

FIGURE 3

Theoretical Concept of Surface Charges Resulting From Ionization of Four Amino Acids Present in Poliovirus Coat Protein at pH Values 5 and 9[1]

[1] Levintow and Darnell, 1960

Amino acid	Net surface charge at pH 5.0	Net surface charge at pH 9.0
Cysteine	CH_2SH	S^-
Histidine		
Lysine	$^+NH_3-R-C_1H-COOH$ NH_3^+	$NH_2-R-C_1H-COOH$ NH_2
Arginine	$^+NH_3-R-C_1H-COOH$ NH_3^+	$NH_2-R-C_1H-COOH$ NH_2

FIGURE 4

Structural Configuration at Octahedral Site

of Fe Atom of Magnetic Iron Oxide

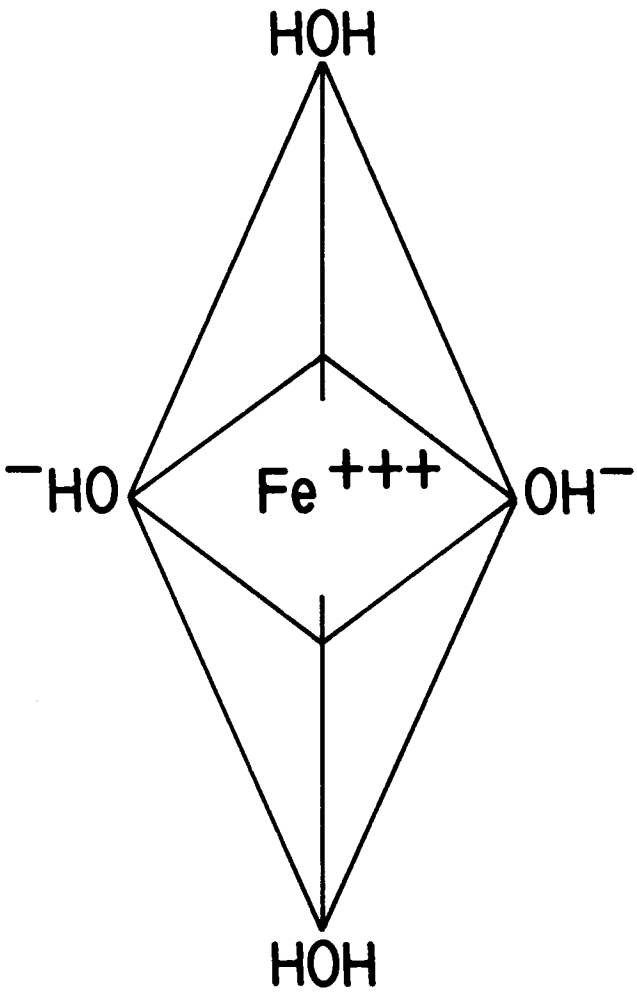
HOH
$^{-}$HO
Fe^{+++}
OH^{-}
HOH

TABLE I

Removal of Poliovirus I By Magnetic Iron Oxide

Trial	Distilled Water (ml)	Virus in Distilled Water ($TCID_{50}$/ml)	Virus in Filtrate ($TCID_{50}$/ml) [1]Remote Site Sampler	[2]Membrane Filter Holder
1	1000	$1.4x10^6$	0	$1.0x10^2$
2	1000	$0.7x10^6$	$2.1x10^2$	0
3	1000	$2.3x10^6$	0	0
4	1000	$0.7x10^6$	0	0
5	1000	$1.3x10^6$	$2.7x10^2$	$1.5x10^2$
6	1000	$1.0x10^6$	0	0
7	1000	$2.2x10^6$	0	$1.4x10^2$

1 Magnetic iron oxide column, 2x4.5 inches.

2 Magnetic iron oxide layer, 3x142 mm.

TABLE 2

Elution of Poliovirus I From Thin Layers of Magnetic Iron Oxide[1]

Trial	[2]Initial Virus ($TCID_{50}$/ml)	Virus in Filtrate ($TCID_{50}$/ml)	Eluate ($TCID_{50}$/ml)		[5]Recovery (%)
			[3]Beef Extract	[4]Eagles MEM	
1	3.09×10^6	0	-	3.1×10^6	25
2	1.7×10^6	0.3×10^6	3.86×10^6	-	69
3	11.1×10^6	0	3.6×10^7	-	81
4	0.6×10^6	0	-	7.2×10^5	30
5	4.5×10^6	0	-	4.86×10^6	27
6	2.3×10^6	0.2×10^6	7.4×10^6	-	88

1 3x142 mm layers.
2 Virus present based on assay of 1000 ml of distilled water suspension passed through iron oxide.
3 250 ml of 3 percent Difco Beef Extract, pH 8.
4 250 ml of Eagles MEM with 10 percent fetal calf serum, pH 8.
5 Percent of initial virus minus virus lost in filtrate.

TABLE 3

Elution of Poliovirus I From Columns of Magnetic Iron Oxide[1]

Trial	[2]Initial Virus ($TCID_{50}$/ml)	Virus in Filtrate ($TCID_{50}$/ml)	Eluate ($TCID_{50}$/ml) [3]Beef Extract	Eluate ($TCID_{50}$/ml) [4]Eagles MEM	[5]Recovery (%)
1	$1.7x10^6$	0	$9.5x10^5$	-	14
2	$2.2x10^6$	0	$4.75x10^6$	-	54
3	$1.4x10^6$	0	-	$5.0x10^5$	9
4	$3.1x10^6$	0	-	$8.68x10^5$	7
5	$5.6x10^6$	0	-	$6.72x10^5$	3
6	$1.3x10^6$	$0.5x10^6$	$1.24x10^6$	-	39

1 2x4.5 inch columns.
2 Virus present based on assay of 1000 ml of distilled water suspension passed through iron oxide.
3 Virus recovered in 250 ml of 3 percent Difco Beef Extract, pH 8.
4 Virus recovered in 250 ml of Eagles MEM with 10 percent fetal calf serum pH 8.
5 Percent of initial virus minus virus lost in filtrate.

TABLE 4

Enterovirus Recovered by Elution Following Passage of Virus Through Thin Layers of Magnetic Iron Oxide[1]

Trial	Enterovirus	[2]Virus in Water ($TCID_{50}$/ml)	[3]Virus in Filtrate ($TCID_{50}$/ml)	[4]Virus in Eluate ($TCID_{50}$/ml)	[5]Recovery (%)
1	Poliovirus I	$1.4x10^6$	0	$5.0x10^6$	91
2	Poliovirus I	$3.1x10^6$	$0.46x10^5$	$9.65x10^6$	79
3	Poliovirus I	$2.2x10^6$	0	$7.1x10^6$	81
4	Poliovirus 2	$1.7x10^6$	0	$5.9x10^6$	88
5	Poliovirus I	$1.3x10^6$	0	$4.3x10^6$	83
6	Coxsackievirus B3	$5.6x10^6$	0	$1.7x10^7$	78
7	Poliovirus I	$3.1x10^6$	$0.23x10^6$	$8.8x10^6$	85
8	Echovirus 6	$0.17x10^6$	0	$6.3x10^5$	93
9	Poliovirus I	$2.2x10^6$	0	$6.6x10^6$	76
10	Coxsackievirus B3	$3.1x10^6$	0	$1.0x10^7$	86
11	Echovirus 6	$0.23x10^6$	0	$7.6x10^5$	83

1 3x142 mm layers.
2 Initial virus obtained from assay of 1000 ml of distilled water-virus suspension passed through iron oxide.
3 Virus in Magnetic iron oxide filtrate.
4 Virus recovered in 250 ml eluate of 3 percent Wilson's Beef Extract, pH 9.0.
5 Percent initial virus minus virus lost in filtrate.

TABLE 5

Interference by Organic Components of Beef Extract with Virus Recovery by Magnetic Iron Oxide

Trial	Organic Component (3%)	[2]Virus in Filtrate ($TCID_{50}$/ml)	[3]Recovery Interference (%)
1	[1]Virus Control	0	0
2	Beef Extract	5.2×10^6	93
3	[a]Peptone	4.6×10^6	82
4	[a]Proteose peptone	5.0×10^5	9
5	[b]High pK_a amino acids	4.8×10^6	86
6	[c]Creatinine	4.4×10^5	8
7	[d]Basic amino acids	1.1×10^6	21
8	[e]Vitamins	7.8×10^5	14

1 Assay for 1000 ml distilled water-virus suspension = 5.6×10^6 $TCID_{50}$/ml.
2 Virus in magnetic iron oxide filtrate.
3 Percent interference based on amount of virus found in iron oxide filtrate.

a Difco
b Glycine, alanine, valine, proline
c Phanstiehl Chemical Co.
d Arginine, lysine, histidine
e Thiamine, biotin, folic acid, pyridoxine, pantothenic acid, riboflavine, nicotinamide

TABLE 6

Elution of Virus from Magnetic Iron Oxide Induced by Organic Substances with Functional Amino Nitrogen Groups[1]

Trial	Organic Substance (3%)	[2]Virus in Eluate ($TCID_{50}$/ml)	[3]Elution Effectiveness %
Theoretical recovery calculated		$1.24x10^7$	100.0
1	High pK_a amino acids	$1.4x10^6$	89.0
2	Spermine	$1.1x10^5$	0.9
3	EBSS with 2% FCS	$1.16x10^7$	94.0
4	Uracil and Xanthine	$2.0x10^5$	13.0
5	Glucosamine	$1.9x10^5$	1.6
6	Glucose	0	0
7	Streptomycin	0	0

1 1000 ml virus suspension with assay of $3.1x10^6$ $TCID_{50}$/ml passed through magnetic iron oxide. No virus found in filtrate.
2 Virus assay for 250 ml eluate.
3 Elution effectiveness determined by comparison of eluate virus assay with magnetic iron oxide virus. 100 percent virus recovery in eluate calculated as $1.24x10^7$.

a Glycine, alanine, valine, proline.
b Obtained in purified form from Dr. E. Herbst, Biochemistry Department, University of New Hampshire.
c Earles balanced salt solution with 2 percent fetal calf serum.
d Uracil as 2,4-dioxopyrimidine, Xanthine (sodium) and D-Glucosamine, N-Acetyl were obtained from General Biochemicals
e Streptomycin sulfate, Eli Lilly & Co., 60 mg/ml

TABLE 7

Amino Acid Functional Groups Involved in Hydrogen Bonding[1]

DONORS	ACCEPTORS
amide hydrogen	amide carbonyl oxygen
carboxylic acid	carboxylic acid and carboxylate
OH (serine, threonine)	OH
ammonium	amino
phenol	phenolate and phenol
quanidinium	quanidate
imidazolium	imidazol
sulfhydryl	sulfide

1/ Repreinted from Introduction to Biophysical Chemistry by R. Bruce Martin, McGraw-Hill Book Co., New York, N. Y.

TABLE 8

The X-ray Diffraction Powder Pattern of Magnetic Iron Oxide

Line	[1]Relative Intensity	[2]Diffraction Angle 2θ	dÅ	[3]Gamma Fe_2O_3 dÅ	Cubic Fe_3O_4 dÅ
1	W	19.0	5.87	5.95_6	
2	W	23.2	4.82	4.84_4	4.85_4
3	W-M	30.0	3.74	3.75_x	
4	W	32.8	3.43	3.42_7	
5	W	34.6	3.26	3.22_3	
6	$M\text{-}S_3$	38.4	2.95	2.95_{x^+}	2.97_7
7	M_4	40.8	2.78	2.80	
8	S_1	45.2	2.52	2.52_{x^+}	2.53_x
9	VW	47.2	2.42		2.42_1
10	W	49.8	2.30		
11	M_4	55.4	2.08	2.09_{x^+}	2.10_7
12	VW	62.0	1.88		
13	VW	64.2	1.82	1.82_2	
14	W	66.0	1.78		
15	W-M	69.0	1.71	1.70_{x^+}	1.71_6
16	W	72.8	1.63		
17	$M\text{-}S_3$	74.2	1.61	1.61_{x^+}	1.61_8
18	S_2	82.0	1.48		1.48_8

1 Values recorded as weak (W) medium (M) or strong (S). Subscript number indicates intensity rated on scale of 1 (least) to 4 (greatest).

2 dÅ- spacing of crystal plane in angstrom units.

3 Subscript number indicates percent rated from 10 (1) through 100 (x). X^+ indicates value greater than 100.

TABLE 9

Elements Other than Iron Found in Gamma Spinel of Magnetic Iron Oxide

ELEMENT	RELATIVE AMOUNT PRESENT (parts per million)
Silicon	Moderate
Chromium	Moderate
Cobalt	Large
Copper	Moderate
Manganese	Large
Nickel	Large
Zinc	Large
Molybdenum	Large