in per cent ash was due to a stimulated production of organic matrix. If this were correct, it could then be suggested that vit. D_3 deficiency in the chick inhibited either the synthesis or physiological activity of ascorbic acid.

Summary. Subcutaneous injection of ascorbic acid given to vit. D_3 deficient chicks resulted in a highly significant reduction in per cent bone ash suggesting that the introduction of this compound had stimulated bone resorption. Calcium and phosphate release by incubating bone tissue did not support this idea, although the movement of hydroxyproline did. An alternative explanation is based on the possibility that the marked change in per cent bone ash resulted from a change in the organic-inorganic ratio of bone tissue. If this were true, it would mean that the ascorbic acid stimulated matrix formation had but little or no effect on calcification.

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Received January 3, 1966. P.S.E.B.M., 1966, v121.

Adsorption of Myxoviruses on Magnetic Iron Oxides. (31020)

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While investigating the phagocytosis of iron oxide particles by cell cultures, it was observed that addition of powdered Fe_2O_3 to allantoic fluids containing each of several myxoviruses caused a decrease in the infective titer of the supernatant and the virus was found to adhere to the rapidly settling metallic oxide. By placing a magnet in the proximity of the bottom or sides of a glass vessel, it was a simple matter to collect the magnetic oxide on the lower surfaces and remove the supernatant fluids. The procedure could be repeated at will to wash the virus. This did not destroy infectivity, and, depending upon the agent used, loss of virus or antigen from the iron oxide was negligible. When it was found that separation of concentrated viral antigen from the complex could be readily accomplished by appropriate treatment, this reaction became one of general interest and is described below.

Materials and methods. Iron oxide. Powdered, acicular or cuboidal Fe_2O_3 (hematite) was obtained from the C. K. Williams Division of Chas. Pfizer & Co., Inc. It was further pulverized by grinding in a ball mill with distilled water for 4 hours to an average particle size of 0.5-1 μ. The final thick slurry was centrifuged to remove excess water and an aliquot dried at 100°C for 20 hours to determine dry weight. Stock suspensions of the wet slurry containing 50 mg/ml were made in distilled water and autoclaved to sterilize. Resuspension of dried Fe_2O_3 powder in water was very difficult and pipetting of aqueous iron suspensions must be performed rapidly in order to secure even distribution of the rapidly settling material.

Viruses. Six representative, egg-adapted strains of influenza virus were grown in the chorioallantoic fluids of 9-day-old embryonated eggs for 48-72 hours and the freshly

TABLE I. Adsorption of Influenza Viruses to Fe_2O_3.

Strain	Virus concentration in original*	Virus concentration remaining in supernate after adsorption
PR 8	960	40
B/Lee	512	256
B/England	128	8
Swine	256	4
Jap 305	256	4
Ann Arbor	128	1

* Hemagglutinating units/ml.

harvested allantoic fluids clarified by light centrifugation. These materials were used either fresh or after prolonged periods of storage at −70°. The hemagglutination titers of these strains were determined by conventional methods, using chick erythrocytes and incubating the mixtures at 23-25°C for 45 minutes before reading. In certain experiments, inactivated virus was prepared by addition of 1:4,000 formaldehyde to the infected allantoic fluids and incubating at 37°C for 8 hours.

Results. Adsorption of virus to Fe_2O_3. Two hundred mg of iron oxide was added to tubes containing 10 ml of allantoic fluid suspensions of the strains of influenza virus shown in Table I and the mixtures gently agitated on a rotary shaker for 30 minutes at room temperature. The tubes were then placed upright between the poles of a large "U" magnet resulting in rapid settling of the iron and permitting decantation of the supernatant fluid when the tubes were inverted with the base held between the poles. The concentration of residual virus in the pour-off fluids was reduced to a degree which varied between viruses. The Lee strain of B influenza was consistently adsorbed less effectively than each of the others, but in subsequent experiments it was found that an optimal amount of iron oxide was required for maximal removal. Table II illustrates the results of one such experiment with PR8 virus, and it will be seen that 20 mg/ml of iron oxide removed essentially all hemagglutinating activity from the supernate. The virus titer in a sodium phosphate eluate (see below) is also shown. The efficiency of adsorption of PR8 virus was not influenced by pH between 5.5 and 8.0 but at a pH of 8.5 or greater there was a reduction in the adsorption efficiency of the iron. Elevating the temperature of the mixture to 37°C increased by 2-4-fold the amount of virus adsorbed to sub-optimal concentrations of iron oxide. Fe_2O_3 particles were not macroscopically agglutinated by any of these 6 strains of myxovirus at 25 or 37°C. The infectivity of virus for embryonated eggs was not reduced by contact with Fe_2O_3 for at least 24 hours at 5°C.

Elution of myxovirus from magnetic iron oxide. Because the metal-virus reaction provides a simple and rapid method for concentration of susceptible virus particles, some effort was expanded to find ways of breaking the complex and recovering viral antigen. Suspensions of virus-coated iron oxide particles in borate, citrate or veronal buffers, various quaternary ammonium wetting agents or hypertonic solutions of sodium chloride, acetate or glutamate failed to dissociate the viral antigen. However, when virus-iron oxide complexes were shaken with a saturated aqueous solution of sodium carbonate ($NaHCO_3$) or 10% sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) for 15 or 20 minutes at room temperature, all of the adsorbed HA antigen could be recovered in the supernate after the iron oxide was brought down by a magnet or light centrifugation. The results of experiments employing PR8 virus are shown in Table III where it can be seen that essentially quantitative recovery was obtained in a 5× concentrate. Each of the 6 influenza viruses which we have studied behaved in a similar fashion. Using mixtures of 10% disodium phosphate and monosodium phosphate to obtain the desired pH ranges, there

TABLE II. Determination of Optimal Amount of Fe_2O_3 for PR-8 Adsorption.

Iron oxide (mg/ml)	Virus conc in adsorption supernate*	Virus conc in eluate
0	960	—
1.0	640	480
2.5	480	640
5.0	160	640
7.5	120	960
10	40	960
15	20	960
20	4	960
40	2	640

* HA/ml.

TABLE III. Elution of PR-8 Virus from Fe_2O_3 with Phosphate or Carbonate.

	Virus concentration*
Elution with $Na_2HPO_4 \cdot 7\ H_2O$	
Original	960
Adsorption supernate	30
5× conc extracts in Na_2HPO_4	3840
Elution with $NaHCO_3$	
Original	**960**
Adsorption supernate	30
5× conc extract in $NaHCO_3$	5180

* HA/ml.

TABLE IV. Effect of pH on Removal of PR-8 Virus from Iron Oxide with 10% Sodium Phosphate.

	Virus concentration*
Original virus, pH 7.4	960
Adsorption supernate	40
Eluate at pH 6.0	640
" " pH 6.5	960
" " pH 7.0	960
" " pH 7.5	1920
" " pH 8.0	1280
" " pH 8.5	1920
" " pH 9.1 (Na_2HPO_4 only)	1280

* HA/ml.

was a pH optimum for elution lying between pH 7.5 and 8.5 for PR8 virus as shown in Table IV. These were not determined for other strains. The data suggests that some disaggregation may have resulted since the HA titer of the eluates, when diluted to original volume, exceeded that of the starting material at pH greater than 7.5. Both infectious or inactivated influenza viruses could be adsorbed and eluted in a similar fashion. Ten percent phosphate solutions had no deleterious effect on influenza HA antigens and the 10× concentrates of 5 influenza strains underwent no decrease in HA activity when stored at 5°C for as long as 7 months.

Antigenicity of influenza virus concentrated by iron oxide adsorption and elution. Commercial influenza vaccines are usually concentrated and partially purified by centrifugation, by protamine precipitation, or by adsorption and elution from a salt such as calcium phosphate or aluminum oxide. Because iron oxide adsorption is a simple and economical procedure, it was of interest to prepare an experimental polyvalent influenza vaccine by this method and determine its capacity to stimulate antibody in the guinea pig.

Freshly harvested allantoic fluids from eggs inoculated with the strains shown in Table V were inactivated with formaldehyde and then adsorbed to iron oxide by the general procedures described above. The supernatant fluids were discarded and the iron oxide-virus complex washed twice with physiological saline solution. The virus was then eluted from the complex with sodium phosphate to a final volume which represented a 10-fold concentrate of the original allantoic fluid. These materials were then titrated for HA activity. Equal portions of the preparations were then pooled and the HA titer of the mixtures again measured. Groups of 15 guinea pigs were vaccinated intramuscularly with a single 0.5 ml dose of the original untreated allantoic fluids and with the vaccines after adsorption on iron and elution and concentration in sodium phosphate. Three weeks after vaccination, the animals were bled and the hemagglutination inhibition titers of their sera determined against standardized preparations of each of the 5 viruses used in the vaccines. As can be seen from Table V, the recovery of antigen in the 10× concentrates was usually less than the theoretical and the best concentration was obtained with the Ann Arbor and PR8 strains. Nevertheless, each of the concentrated vaccines stimulated increased levels of HAI antibody when compared with the original starting vaccines. Overall losses from this procedure compare favorably with those encountered when influenza viruses are concentrated by Sharples centrifugation. The nitrogen concentration of each of these preparations was determined before and after adsorption of the virus on iron oxide and its elution. From the results shown in Table V, it is apparent that this treatment results in the removal of a considerable amount of nonantigenic nitrogen.

Discussion. Using a highly purified gamma ferric oxide prepared by the methods of Baudisch(1), phagocytosis of magnetic ferric oxide by Kupfer cells and their subsequent isolation from liver tissue in a magnetic field was described by Rous and Beard in 1934

TABLE V. Concentration of Influenza Antigens with Ferric Oxide and Antibody Response in Vaccinated Guinea Pigs.

Vaccine	Ann Arbor	PR-8	Jap 170	B/Md	B/Lee
Orig allantoic fluid	640	2560	160	80	320
" " " —10× conc	5120	32000	640	640	2560
Conc based on HA	8×	12.5×	4×	8×	8×
HA units/0.5 ml dose					
Orig allantoic fluid	128	512	32	16	64
" " " —10× conc	1024	6400	128	128	512
Total nitrogen-μg/ml					
Orig allantoic fluid	654	609	693	610	620
" " " —10× conc	109	58	81	24	44
Antibody response of guinea pigs (G.M.T.)					
To orig allantoic fluid	3.2†	8.2	5.0	2.5	1.8
" " " " —10× conc	26.3†	42.6	15.2	13.7	6.7

* HA/0.5 ml. † HAI—reciprocal of serum dilution.

(2). These authors also noted the lack of toxicity of ingested iron oxide particles when the isolated Kupfer cells were subsequently cultivated *in vitro*. We have found no reports of viral adsorption to Fe_2O_3.

The attraction of myxoviruses to iron oxide appears to be a physical phenomenon which does not produce any detectable biochemical alterations in the virus particle. The magnetic iron-virus complex can be removed from solution by continuous flow over a magnetic field or by attraction from a batch or slurry. The sodium phosphate and sodium carbonate concentrations required to separate the complex do not have a deleterious effect on myxoviruses; their action on other agents is not known to us. Preliminary experiments with poliovirus resulted in considerable removal of this agent on the surface of iron oxide, suggesting that microorganisms other than myxoviruses will also be adsorbed to Fe_2O_3.

Summary. Influenza virus will adsorb to powdered magnetic ferric oxide and can be eluted from the iron with appropriate concentrations of sodium phosphate or sodium carbonate. This reaction appears to be a physical adsorption and provides a simple method for concentration and partial purification of viruses.

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Received January 3, 1966. P.S.E.B.M., 1966, v121.

Some Ion Exchange Properties of a Myelin Extract from Bovine Optic Nerve.* (31021)

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Several methods have recently been reported for isolation of a myelin-like or myelin enriched extract from nerve(1-4). Inasmuch as myelin is probably some extension of Schwann cell or glial plasma membranes its isolation in bulk should afford a cell membrane system readily amenable to direct study. By inference information has been

* This work was supported in part by a grant from USPHS and in part by a grant from Dr. Wallace C. and Clara A. Abbott Memorial Fund of University of Chicago.

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