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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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

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gathered about the ion exchange properties of myelin by comparing the ion and water content of gray and white matter and studying these at various stages of myelination of the developing brain(5,6). Several workers have observed that not all the cation content of brain homogenates could be removed by dialysis(6-8) and this has led to the consideration of cation binding by acidic components of the nervous tissue. Katzman and Wilson (9), using a rapid freezing method to extract brain lipids at —45°C and thereby decrease the exchange between lipid and inorganic cations, found the extracted cations in combination with the phosphatidyl serine. While attempts are in progress to define the specific affinities between particular cations and particular acidic lipids from brain(10-12) the situation with regard to intact segments of the stable bimolecular lipid leaflets and their counterion associations may be quite different from those of the isolated pure lipids. The work reported here was begun with this in mind but with the reservation that the extracted material was probably neither pure nor identical with intact native myelin.

Methods. The extraction method used here was that of Patterson and Finean using sucrose(1) but with the addition of 2 steps involving the suspension of the extract in water to release entrapped axoplasm and inclusions by osmotic shock. Pellets of the sedimented 'myelin' were dispersed in solutions containing 500 mEq/l of $CaCl_2$, NaCl or KCl and allowed to stand at 4°C for 24 hours, sedimented again and dispersed in soaking solutions containing varying concentrations of the same salts, and allowed to stand in these for 72 hours at 4°C. The 'myelin' preparations so treated were sedimented at 20,000 *g* at 4°C for 30 minutes, and the water and ion contents of the pellets determined, the former by weight loss on drying at 80°C to constant weight, and the latter by standard ashing and flame photometric procedures.

Results and discussion. The pellet water content for the Ca, Na and K forms of the 'myelin' decreased with increasing concentration of the soaking solution over the range 0 to 500 mEq/l, and at every equivalent concentration used was significantly less for the Ca than for either the Na or K forms. There was no difference in the pellet water content between the Na and K forms exposed to the same concentration of soaking medium.

Over the soaking solution concentration range of 0-500 mEq/l of NaCl, KCl or $CaCl_2$ the extract pellet water cation concentration was in excess of that of the soaking medium by between 0 and 15 mEq/l. In no case was the pellet water cation concentration less than that of the medium. This pellet water and ion content behavior was similar to that of the weakly cross-linked cation exchange resins(13). It was concluded that the 'myelin' carried a net negative charge at the pH of these experiments, which was about 6.0, as might be expected from the acidic lipid content of myelin(14,15).

It was not possible to remove all of the Ca from the extract by soaking in NaCl or KCl for 72 hours. For this reason a study was undertaken to determine some of the properties of the exchange processes involved in determining the amount of this residual Ca. To do this the 'myelin' extract was first loaded with Ca by soaking for 24 hours in 500 mEq/l $CaCl_2$ and it was then subsequently exposed to various concentrations (30-50 mEq/l) of LiCl, NaCl, KCl, RbCl or CsCl for 72 hours, all steps being done at 4°C. The Table shows the effects of these salt solutions on the residual Ca content of the 'myelin.' Three effects were observed. 1) The extract residual Ca was largest at highest soaking solution concentrations. 2) The smaller the hydrated diameter of the exchanging monovalent cation the better it exchanged with the Ca. 3) The higher the soaking medium concentration the greater was this discrimination between the monovalent cations.

Soaking the 'myelin' in 100 or 500 mEq/l $MgCl_2$ for 72 hours resulted in mean residual Ca contents of 4.6 and 5.5 μM/100 mg dried extract respectively. Magnesium therefore appeared to be of the same degree of effectiveness as Cs in its ability to exchange with Ca. Due to the effect of cation valence on myelin chemistry(16) it is difficult to evaluate the Ca-Mg exchange with reference to the Ca-monovalent cation exchanges.

TABLE I.

Exchange solution	Residual calcium content (μM/100 mg dried extract) Concentration .50M	.30M	.10M	.05M	.01M
LiCl*	8.2†	6.8	5.6	4.4	—
NaCl	7.1	6.0	5.6	3.8	3.1
KCl	6.5	6.1	5.2	4.1	3.5
RbCl	—	6.1	4.8	3.9	—
CsCl	—	5.2	4.6	4.2	—
Differences‡	Li > Na $p < 0.01$	Li > Cs $p < 0.01$	Li > Cs $p = 0.04$	Li > Cs $p = 0.6$	

* Each lot of extract was divided into 5 fractions and one of each of these soaked in the various exchange solutions of the same concentration.

† Each number is the mean calcium content of at least 5 separate lots of extract.

‡ Statistical evaluation was performed by t test of paired experiments from the same lot of extract.

The observation that the residual Ca is highest at highest soaking solution concentrations may be due to an ionic strength dependent chemistry of the extract. For example LeBaron and Folch(17) found that varying the ionic strength caused varying amounts of protein to be extracted from nervous tissue, though whether myelin protein was affected was not absolutely determined. Due to the variability of each lot of extract it was necessary first to soak each lot in $CaCl_2$ and then divide them into 5 fractions for exposure to exchanging solutions of the same concentrations. As the sequence of exposures was such that the same lot of extract was exposed to the same ionic strength, though different monovalent cations, it was felt that the monovalent cation discrimination was not due to any alteration of 'myelin' chemistry.

From the Table it would appear that the monovalent cation discrimination sequence seen here was on the basis of the ion's diameter (crystal or hydrated) and that the magnitude of the discrimination was concentration dependent. In recent years there have been several theoretical approaches advanced to account for the various ion discrimination sequences seen in biological systems (*e.g.*, 18). Vandenheuvel(19), considering a myelin model in which a water monolayer is located at the protein layer, finds that the naked ion to best fit into such a water layer with least distortion is K, then Rb, Cs, Na, and Li, a sequence unrelated to that seen here.

The discrimination of monovalent cations by their Ca exchanging ability must be considered in two frames of reference. Myelin is a highly structured material with a water content of only about 50%(20), and this water is probably limited to the hydrophilic areas of the bimolecular lipid leaflets and their associated proteins. For an ion to exchange with one already bound in this material it must first penetrate into the interlamellar water spaces. Having gained access to the exchange site it must then displace the ion already located there, which in this case, being a divalent cation, is probably located at 2 adjacent sites.

Several observations suggest that the spaces available for water in myelin may change in response to a changing osmotic pressure in the environment(21,22). Palmer and Schmitt(23) concluded from low angle X-ray diffraction data on mixed spinal cord lipid emulsions that increasing the emulsion salt concentration reduced the interleaflet water content. Finean and Millington(21) found that the major repeating unit of myelin increased in hypotonic solutions and decreased in hypertonic ones. In this work the greater cation exchange discrimination seen at the higher salt concentrations may be a reflection of the diminished interlamellar water spaces making penetration of the hydrated counterion the limiting factor in the exchange process. This may in part account for the fact that less Ca was exchanged by all species at the higher salt solution concentrations. As the salt concentration is reduced the interlamellar water becomes less restricted and the size of the ion hydration shells of less impor-

tance in determining the number of counterions available at exchange sites. Or in other words, provided the water is not structured around these sites, the lower the salt concentration the greater will be the proportion of free water in the interlamellar spaces and the more easily will a counterion fit into these spaces, regardless of its size.

Summary. The water content of pellets of a myelin extract of bovine optic nerve decreased with increasing salt concentration of the medium in which they were spun down. This water content was always less when Ca was the medium cation than when either equiequivalent Na or K was present. The pellet water cation concentration was always greater than that of the medium for the above 3 cation species. It is concluded that the extracted material carried a net negative charge. Extract Ca could not be completely exchanged by Li, Na, K, Rb, Cs, or Mg. At higher salt concentrations the ease of exchange of Ca by monovalent cations was in the same sequence as their hydrated diameters, the smallest counterions exchanging most readily.

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Thyroxine Biosynthesis and Thyroidal Uptake of I^{131} in Rats at The Onset of Hypoxia Exposure.* (31022)

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Based on early observations that hypoxia tolerance can be increased by thyroidectomy or injections of antithyroid drugs(9,17) there have been sporadic attempts to determine the extent to which hypoxia exposure influences thyroid function in the intact animal. The

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