to 3–6 *M* guanidine (*2*), the outlines of the particles in phosphotungstate are ragged and no membrane structures are seen. On several occasions, however, short chains of "donuts" were visible close to the edge of the particles. These structures were more clearly defined than in urea-treated particles; they measure 20–25 mμ in diameter and have a dark central hole approximately 8 mμ in diameter.

An interesting effect is observed when virus is exposed to 2–4 *M* guanidine (Fig. 1d). The center of the particles becomes filled with phosphotungstate and the capsid is clearly outlined as though in cross section. The capsid is 20–25 mμ across, and within the dark central pool are seen shadowy elongated structures that probably represent the remnants of the nucleoid or the lateral bodies (*6*). The resemblance of these vaccinia particles to "empty" capsids of herpes virus is striking (*4*). The presence of phosphotungstate in the center of guanidine-treated vaccinia confirms the finding that, in section, the central region of such virus has largely disappeared; sections of urea-treated virus, on the other hand, show remarkably good preservation of the nucleoid structures (*7*).

It is apparent that prior treatment of vaccinia virus with various concentrations of reagents such as urea or guanidine, combined with negative staining with phosphotungstate, reveals both surface detail and internal structures that are not seen by conventional shadowing methods or by negative staining alone. Evidence has been obtained that vaccinia, like other viruses such as herpes (*4*) or polyoma (*8*) is surrounded by a clearly defined capsid, which in this case is 20–25 mμ thick; and there is preliminary evidence that the capsid may be composed of regular capsomeres 15–25 mμ in diameter. The capsomeres are presumably distributed uniformly over the viral surface, but so far have been visualized by the electron microscope only close to the edges of the particles. Further investigations of the fine surface structure revealed in reagent-treated vaccinia will be described elsewhere.

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A Simple Procedure for Purification of Viral Hemagglutinin[1]

The statement by Hoyle (*1*) that S antigens of influenza A viruses liberated by ether treatment could be precipitated by lanthanum acetate, suggested the possibility that lanthanum acetate might be used to effect a separation of viral hemagglutinin from viral S antigen.

The results of studies to test that hypothesis are briefly summarized in this communication. Concentrated suspensions of virus were prepared according to a variety of procedures. Virus concentrates were treated at 4°C with three volumes of peroxide-free anesthetic ether on two occasions for a period of 8 hours each. Agitation was accomplished by use of a magnetic stirrer, and separation of the fluid phases by a separatory funnel. Excess ether was removed from the combined aqueous phases by bubbling N_2

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TABLE 1

PARTIAL PURIFICATION OF ETHER-LIBERATED HEMAGGLUTININS BY TREATMENT WITH LANTHANUM ACETATE (LaAc)

Test	Swine	PR8	FM1	A_2 1957
CF, S titer before LaAc	32	128	16	16
CF S titer after LaAc[a]	1	4	2	1
HA titer before LaAc	640	10,240	1280	2560
HA titer after LaAc[b]	320	5,120	640	1280

[a] Uncorrected for dilution with lanthanum acetate.

[b] Corrected for dilution with lanthanum acetate.

Overnight fixation in the cold was routinely employed.

Typical findings observed with swine, A, A prime, and Asian strains are shown in Table 1. The HA titer values found were doubled to compensate for dilution with lanthanum acetate. The CF titers were not so corrected. Anti-S antisera were prepared in guinea pigs using the procedure of Lief *et al.* (*4*). In each case the concentration of S antigen measured serologically was reduced to trace amounts by a single treatment with lanthanum acetate. Electron micrographs confirmed this finding, since the characteristic beaded rods of S antigen (*5*) were clearly apparent in the ether-extracted suspensions, but could not be readily identified in the lanthanum acetate-treated preparations (Fig. 1).

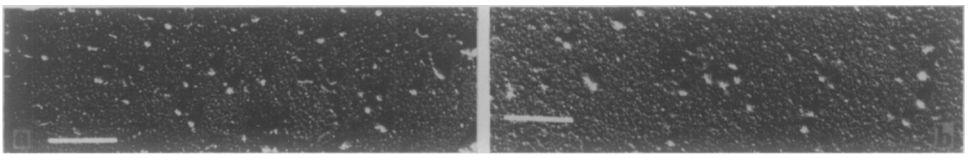

FIG. 1. Appearance before (a) and after (b) sodium acetate treatment.

through that suspension. Dialysis against large volumes of 0.15 *M* sodium acetate pH 7.2 was then carried out for 48 hours at 4°C.

To remove S antigen, equal volumes of the dialyzed ether-treated suspensions were mixed with a 1 or 5% freshly prepared solution of lanthanum acetate in 0.15 *M* sodium acetate buffer pH 6.5. After standing for 2 hours at 4°, the mixture was centrifuged at low speed in a clinical centrifuge for 30 minutes. The supernate was removed and to prepare it for testing was dialyzed either against 0.25 *M* sodium citrate pH 7.4 if hemagglutinin only was to be measured, or against 0.25 *M* sodium citrate, followed by 0.15 *M* sodium acetate pH 7.2, if complement fixation determinations were to be made.

Titrations of hemagglutinins (HA) were performed with chicken erythrocytes according to a standard procedure (*2*). Complement fixation (CF) titrations using 2 units of complement were conducted by the micro method of Fulton and Dumbell (*3*).

The reason for the drop in hemagglutination titer is unclear. Significant amounts of hemagglutinin were not found in the precipitate, which contains the bulk of the S antigen. Aggregated hemagglutinin was not seen in the electron micrographs. The drop in titer was not always found or was slight in many experiments. The results were similar in preliminary studies with zirconium instead of lanthanum salts.

One objective of this investigation is to develop simple chemical and physical procedures for the isolation and purification of the hemagglutinin liberated by ether which might be useful in the manufacture of influenza virus vaccines. The potential usefulness, for other purposes, of the reactions described herein prompted this early report.

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Density Gradient Centrifugation of Bacteriophage P22

An investigation of defective, high frequency transducing (HFT) particles in λ lysates (*1*) indicated that independently isolated HFT clones had distinctive densities, different from that of normal λ phage and from one another. In low frequency transducing (LFT) lysates, galactose-positive transducing particles were found to have a greater density heterogeneity than infective particles (*2*). Whereas phage λ participates in transduction only of galactose genes, other phages (e.g., P1 and P22) participate in "generalized transduction" of many bacterial genes (cf. *3*).

HFT lysates of phage P22 are not yet available. However, it was of interest to determine whether transducing particles for different genetic markers in a lytically derived lysate, active in low frequency transduction, could be separated from infective particles and from one another in a cesium chloride density gradient. Also of interest were transducing particles grown on multisite mutants. According to genetic (*4*), enzymatic (*5*), and ultraviolet (*6*) and nitrous acid (*7*) inactivation data, some multisite mutants may have lost considerable amounts of genetic material. Estimates of the size of one bacterial gene region (*6, 7*) made it seem possible that particles containing the mutant region might be separated from transducing particles containing the same bacterial gene region in wild-type form, if the multisite mutations were true deletions and not other types of genetic aberrations and if transducing genomes were otherwise uniform with respect to their content of bacterial (*8*) and phage genes.

High titer stocks (7×10^{11} to 2×10^{12} phage per milliliter) of wild-type P22 phage were grown on wild-type *Salmonella typhimurium* strain LT-2 and purified by treatment with Worthington ribonuclease and deoxyribonuclease, differential centrifugation, and resuspension in saline. A cesium chloride solution was prepared by adding 7.55 ml Difco-nutrient broth to 6.3 g cesium chloride and 0.366 g magnesium chloride. Next, 0.3 ml of the phage stock was added to 3.0 ml of the cesium chloride solution. The mixture was spun at 27,000 rpm for 18 hours in a Spinco model L preparative centrifuge with the swinging-bucket rotor No. SW-39. Single-drop fractions were collected from the bottom of the centrifuge tube after puncture with a No. 24 syringe needle. The fractions were diluted with T2 buffer (*9*), sterilized with chloroform, and assayed for infective centers and transducing particles. Ninety per cent of the infective center input and 48–102% of the transducing particle input (depending on the recipient strain used) were recovered from the gradient.

Activities for complete transductions to wild type both of *hisF-42*, a single-site mutation, and of *hisE,F,A,H,B,C,D-712*, a multisite mutation extending over more than 75% of the known histidine region (*10*) were found to exhibit maximal activity coincident with the peak of infective centers (Figs. 1, 2). This identity in the positions of the peak activities was also found for two separately prepared phage stocks. The peak of infective centers for P22 was coincident with that for T4 *rII* BO^1R (osmotic shock resistant) bacteriophage, assayed from the same centrifuge tube. A mean bouyant density value of 1.45 g/ml has been determined for T4 *rII* BO^1R (*11*). Complete transductions to wild type of *athC-5*, an adenine and thiamine mutant (*12*), were found to exhibit a peak one fraction more dense than did the histidine transductions, whereas complete