TABLE III.
Results of Cold Agglutination and Streptococcus No. 344 Agglutination Tests on Sera from 87 Patients with Primary Atypical Pneumonia.

Group	Cold agglutination	Streptococcus No. 344 agglutination	Civilian	Military	Total
I	Positive	Positive	30	6	36
II	"	Negative	14	9	23
III	Negative	Positive	1	2	3
IV	"	Negative	4	21	25
Total			49	38	87

Cold agglutination positive 68%.
Streptococcus No. 344 agglutination positive 45%.

only with fresh serum,[3] the group on which both tests were done was smaller and different in composition. Forty-nine sera were taken from civilian students and 38 from patients in two military establishments. Thirty-six of 39 persons showing streptococcal agglutination also had cold agglutinins. Twenty-three others had cold agglutinins, but no streptococcal agglutinins. Only 3 showed streptococcal agglutinins without cold agglutinins. The proportion negative by both tests was considerably higher in the military group.

Comment. The composition of the group studied differs from that found in most reports on primary atypical pneumonia in that the proportion of moderately severe and severe cases is unusually high. This may account, in part, for the rather high proportion of positive results. In this group of patients the cold agglutination test appeared to be a more sensitive indicator.

The results reported here do not establish the etiological relationship of an indifferent streptococcus to primary atypical pneumonia. This is in accord with the opinion expressed by Thomas and his coworkers.[1] A virus has been isolated from patients with atypical pneumonia,[4] and persons having agglutinins for streptococcus 344 usually have neutralizing antibodies for this virus; but many patients having an increase in neutralizing antibodies associated with atypical pneumonia have no streptococcal agglutinins. The details of these latter observations will be published in a subsequent report.

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[3] Meiklejohn, G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 181.

[4] Eaton, M. D., Meiklejohn, G., and van Herick, W., *J. Exp. Med.*, 1944, **79**, 649.

14811 P

Cytoplasmic Particles of Chorio-allantoic Membrane and Their Relations to Purified Preparations of Influenza Virus.

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Ultracentrifugally sedimented preparations of Rous sarcoma virus contain antigenic groupings similar to that of the similarly sedimentable normal tissue components.[1] It would seem desirable that other newly isolated viruses be routinely examined for their content of groupings common to the tissue from

[1] Kabat, E. A., and Furth, J., *J. Exp. Med.*, 1940, **71**, 55.

which they have been derived. The demonstration of apparent physical homogeneity and unique particle size is not sufficient to warrant the assumption of alien chemical uniqueness implicit in the designation of "virus" to the isolated body. This is so because it may be expected that a stage in the synthesis of a virus would require the union of the stimulating virus particle and the protein-synthesizing mechanisms of the host. Thus the liberated particle bearing virus activity may also contain considerable portions of the host, the combination possessing new physical properties. In any case, it may be assumed that the orientation of the chemical work on these virus preparations would be affected if it became clear that a considerable portion of the isolated entity was common to a non-infected host. Virus preparations of influenza* were used as a model for an immunochemical assay, since so much chemical, physical, and biological data have been published on purified preparations.[2,3,4] The virus in the chorioallantoic fluid presumably is liberated into the fluid by the cytolysis of infected cells of the allantoic sac.

The chorioallantoic membranes of normal 11- to 13-day-old chick embryos were rinsed frequently in cold saline containing 0.01% merthiolate and were finely suspended in a final volume of 1 cc per membrane. The suspension was centrifuged at 4000 RPM for 1 hour and the resulting supernate at 30,000 RPM for 20 minutes. The pellets were permitted to soak at 4°C in 0.1 M borate buffer at pH 7.8 for 18 hours and emulsified. The differential centrifugation cycle was repeated and the pellets emulsified in buffer and centrifuged at 4000 RPM to yield an opalescent yellowish solution. A solution at 0.5% concentration was kindly examined in the analytical ultracentrifuge by Dr. M. A. Lauffer of the Rockefeller Institute. The particles, NP, yielded a somewhat diffuse boundary, whose peak had an S_{20} of about 245.† The dialyzed dried particles contained 8.00% nitrogen and 1.76% phosphorus. The average yield per membrane was .05-.06 mg nitrogen in 6 experiments involving 460 membranes.

Rabbit antisera to these materials were found to have fairly high titers of Forssman and particle specific antibodies. The strongest serum was standardized quantitatively by the following method:

The particles, dried over P_2O_5 *in vacuo*, were found to be completely insoluble. Emulsified in saline and placed in normal rabbit sera, these suspensions were quantitatively sedimentable at 2500 RPM for 1 hour. After 2 washings with cold saline, the nitrogen content of the pellet, after a small correction for residual serum nitrogen, was identical with the nitrogen content of NP added. The dried preparations of influenza viruses behaved similarly.

A portion of the antisera to NP was absorbed with sheep red cells. One cc aliquots of saline and of NP suspensions of nitrogen content of .025-.25 mg per cc were added to 1 cc aliquots of normal rabbit sera, the unabsorbed antiserum to NP, and the sheep cell absorbed antiserum. The tubes were frequently mixed during incubation at 37°C for 2 hours and at 4°C overnight. The tubes were centrifuged in the cold at 2500 RPM for 1 hour and the pellets were washed twice with two 1.5 cc aliquots of cold saline. The nitrogen contents were determined and 2 nearly linear curves were obtained when the antibody combined with NP was plotted against antigen nitrogen added. Thus 0.250 mg of NP nitrogen combined with 0.150 mg of antibody nitrogen in the unabsorbed serum and 0.115 mg of antibody nitrogen in the absorbed serum, the difference being Forssman antibody.

The agglutination of suspensions of influenza virus preparations, dialyzed and dried as in the above, was then studied. The preparations were of 3 types: the first was a

* The numerous biological materials were kindly supplied by Dr. W. Henle and Dr. G. Henle of the Children's Hospital of Philadelphia.

2 Taylor, A. R., Sharp, D. G., Beard, D., Beard, J. W., Dingle, J. H., and Feller, A. E., *J. Immunol.*, 1943, **47**, 261.

3 Stanley, W. M., *J. Exp. Med.*, 1944, **79**, 255.

4 Friedewald, W. F., and Pickels, E. G., *J. Exp. Med.*, 1944, **79**, 301.

† Although this S_{20} is less than one-half that reported for any influenza strain, it is very close to that obtained for equine encephalomyelitis virus from infectious allantoic fluid.

TABLE I.
Estimates of Content of Normal Membrane Particles in Preparations of Influenza Virus.

Virus preparation	Estimations with antisera for normal particles: Unabsorbed %	Sheep cell—absorbed %
Multivalent formol vaccine	35	27
PR8	51	57
Chick cell absorbed PR8	29	39

formol-treated preparation of multivalent vaccine, prepared by the ultracentrifugation of the infectious chorioallantoic fluids of the fertile hen's egg, and kindly supplied by Dr. W. M. Stanley of the Rockefeller Institute; the second was a preparation of the PR-8 strain isolated by several differential centrifugation cycles and the third was a preparation of the PR-8 strain concentrated by adsorptions and elutions from chick red blood cells and finally sedimented in the ultracentrifuge.

Saline suspensions were added to the 3 sera employed previously. Absorbed antibody nitrogen was determined, referred to the curves previously estimated, and equivalent NP nitrogen values were obtained. The content of normal allantoic membrane particles was then estimated on the assumption that the normal groupings in the virus preparations were as reactive as if they were present in the normal particles described. These calculations are summarized in Table I.

If these normal particles containing the same antigen distribution were built into the particle bearing virus activity, these estimates are minimal since some normally reactive groups are probably rendered inactive. A number of other possibilities may also limit the validity of these estimates. However, the qualitative conclusion may be drawn that current preparations of the influenza viruses contain antigens typical of the host in amounts sufficiently large to render dubious the significance of chemical data obtained in the process of characterizing this material as an alien and specific viral agent.

14812 P

Motor Analysis of Anti-Peristalsis in the Descending Colon of Rabbit.

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As far as we are aware, antiperistalsis of the descending colon of rabbit has never been studied by direct inspection, yet this can be readily accomplished. The rabbit is narcotized by the subcutaneous injection of 150 mg sodium barbital per kilo; after 45 minutes the descending colon is exposed by a cut through the linea alba from the ensiform cartilage to the pubis; a segment of the descending colon is selected and prepared by making two cuts about 9 cm apart; these cuts sever 60% to 80% of the gut, opposite to the mesenteric border; the segment and adjacent portions of the colon are milked clear of scybala. Now by introducing dry, normal scybala either at the oral or aboral cut, peristaltic and antiperistaltic waves may be produced. Sluggish motor activity is readily improved by subcutaneous injection of 0.1 to 0.3 mg of physostigmine sulfate per kilo.

Results. The introduction of a dry scybalum 1 to 2 cm into the segment at the oral or aboral end generally produces a peristaltic contraction after a short period of time. If the progress of the scybalum is prevented by a gentle digital compression of the segment, the peristaltic wave of contraction sooner or later relaxes and the formerly relaxed, com-