

TABLE I.
Serological Findings and Virus Isolation from Apparently Normal Contacts.

Case No.	Serological diagnosis influenza A			Virus isolation Egg passage in which agglutination was first observed	Symptoms
	Acute phase titer	Convalescent phase titer	Significant increase antibodies		
1	1:208	1:1450	+	Fourth	Feeling onset of cold
2	1:223	1:1270	+	Neg.	Slight cough
3	*	*	*	Third	Slight coryza
4	1:91	1:315	+	Neg.	Chronic cough
5	1:147	1:16,400	+	Neg.	No symptoms
6	1:447	1:722	—	Fourth	" "
7	1:91	1:2900	+	Neg.	Slight sore throat
8	1:84	1:97	—	Third	Chronic cough
9	1:21	1:356	+	Neg.	Slight cough
10	1:362	1:891	—	Neg.	No symptoms
11	1:132	1:722	+	Neg.	" "
12	1:223	1:1024	+	*	" "
13	1:223	1:1270	+	Neg.	Slight coryza
14	1:223	1:512	—	Third	No symptoms

* Not examined.

ing infection than are the clinical cases.

Summary. By the direct intra-allantoic inoculation of unfiltered throat washings influenza A virus was isolated from 5 of 13 apparently normal contacts during an epidemic of

influenza A. Significant increases in neutralizing antibodies were demonstrated in the sera of 9 of 13 such contacts by means of the red blood cell agglutination inhibition test.

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Agglutination Tests with Streptococcus No. 344 in Primary Atypical Pneumonia.*

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The discovery by Thomas, Mirick, Ziegler, Curnen, and Horsfall¹ that sera of patients convalescent from primary atypical pneumonia frequently agglutinate an indifferent streptococcus, labeled No. 344, has provided yet another method for investigating this disease. The fact that the streptococcus agglutinins

persist for a long time when stored at 4°C has made it possible to investigate sera from patients with primary atypical pneumonia received at this laboratory since the summer of 1941. Results of the tests, together with certain observations correlating laboratory and clinical findings, form the subject of this report.

Methods and Materials. The method described in the original report was followed without modification. Titers of 10 or more were considered significant.

Sera were collected from students admitted to Cowell Memorial Hospital, University of California, Berkeley, and to the University of

* The studies and observations on which this paper is based were supported by the International Health Division of The Rockefeller Foundation in cooperation with the California State Department of Public Health.

¹ Thomas, L., Mirick, G. S., Curnen, E. C., Ziegler, J. E., and Horsfall, F. L., Jr., *Science*, 1943, **98**, 566.

California Hospital, San Francisco; from patients admitted to certain west coast military establishments; and from scattered patients in the San Francisco area. Both early and late specimens were available in most cases. Those specimens taken during the first week of illness were considered acute. Convalescent specimens were collected between the twelfth and forty-fifth days.

Clinical Observations. Cases were classified prior to this investigation on the basis of complete clinical records. The first day on which constitutional symptoms were noted was considered the day of onset. Criteria used in making a diagnosis of primary atypical pneumonia were (1) the history and course of the disease, (2) X-ray evidence of pneumonia, (3) a white blood count of less than 12,000 during the first week of illness, and (4) the absence of large numbers of pneumococci in direct sputum examination. Cases in which only small numbers of pneumococci were seen and in which typing was accomplished only after mouse inoculation were not excluded if the course of illness suggested primary atypical pneumonia rather than pneumococcal pneumonia. Five of 14 patients with cases of this type developed significant titers.

Results of Tests. Sera from 156 patients were tested; 73 of 156 convalescent sera were positive, the titers ranging from 10 to 160, with only 11 being 40 or more. Of 41 persons whose convalescent serum was positive, only one showed a significant titer in a specimen taken during the first week of illness. Control tests were done with sera from 32 patients with upper respiratory infections, 54 with pneumococcal or other bacterial pneumonias, and 42 with tuberculosis. None of these had streptococcal agglutinins except two from persons with tuberculosis, in which titers of 10 were found.

Correlation of Results with Severity of the Disease. In an attempt to explain why some patients developed agglutinins while others, whose clinical picture was similar, did not, the results were analyzed with respect to certain clinical features. Two of these, both reflecting the severity of the illness, appeared to show some correlation. These were (1)

TABLE I.
Correlation of Results of Streptococcus No. 344 Agglutination with Duration of Fever.*

Duration of fever (days)	Positive	Negative	Total	% positive
< 7	7	47	54	13.0
7-12	41	29	70	58.6
>12	25	7	32	78.1
Totals	73	83	156	46.9

* Febrile day defined as one with single oral temperature reading of 99.6°F or higher.

TABLE II.
Analysis of Results of Streptococcus No. 344 Agglutination with Respect to Maximum Temperature.

Max. temp.	Positive	Negative	Total	% positive
<101°F	5	18	23	21.7
101-103°	24	30	54	41.2
>103°	44	34	78	56.4
Totals	73	82	155	47.1

the duration of fever, and (2) the maximum temperature reading. A similar correlation in the case of cold agglutinins has been suggested by others.²

Duration of Fever (Table I). Among patients whose febrile period lasted less than a week, comprising approximately one-third of the total, agglutinins were formed in only 7 of 54 cases. The percentage of positives increased as the length of illness increased. Among those with febrile periods of 12 days or more, 25 of 32 were positive.

Maximum Temperature (Table II). An obvious error enters into this analysis due to the frequent spiking nature of the temperature curve. There appears, nevertheless, to be some correlation. Only 5 of 23 persons whose temperature failed to reach 101°F were positive. On the other hand, of those persons with maximum readings above 103°F, 44 of 78 were positive.

Combined Streptococcus and Cold Agglutination Studies (Table III). Since cold agglutination tests can be done satisfactorily

² Finland, M., Unpublished data presented at General Meeting of the Society of American Bacteriologists, New York, N.Y., May 3, 1944.

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.

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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.