phoretic patterns. The values were: for pattern 1, 0.7%; pattern 7, 0.7%; pattern 8, 0.7%; pattern 9, 0.8%; pattern 10, 0.8%. Thus there was no significant difference, in this respect, between the plasmas of several kinds of abnormal semens and that of a normal specimen. The refractive indices of the several protein components were assumed to be the same and equal to that of serum globulin.

Summary. Electrophoretic examination of the plasmas from specimens of human semen which were either (1) abnormally viscous or in which (2) the sperm were either poorly motile or non-motile, or which (3) contained no sperm revealed no definitely significant deviation in protein components from normal specimens. Components P1 (proteose), P2 and P3 (globulins) were present in all these specimens as they are in normal ones. Component P2a (globulin), described in an earlier report as being present in occasional normal specimens, was found in some of the abnormal ones also. Normal specimens contain either P4 or P5 (glycoprotein) or both. Component P5 was present in all the abnormal specimens. The absence of P4 from all but one of these is noteworthy, but the varied nature of the abnormality makes interpretation difficult.§

§ We wish to record our appreciation to Dr. John MacLeod and Dr. Robert S. Hotchkiss of Cornell University Medical College for the semen specimens.

14357

The Cold Agglutination Test in the Diagnosis of Primary Atypical Pneumonia.*

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Since February, 1943, when Peterson, Ham, and Finland[1] reported on cold autohemagglutination in primary atypical pneumonia and suggested that this procedure might differentiate the primary atypical pneumonias from other types of pneumonia, all serum specimens coming to this laboratory from patients with respiratory diseases have been tested for cold agglutinins. The chief purposes of this investigation have been (1) to determine how frequently significant titers of cold agglutinins are found in primary atypical pneumonia and (2) to determine how often similar titers are encountered in other respiratory diseases.

Materials and Method. 155 serum specimens from 74 patients diagnosed as primary atypical pneumonia were tested. These came from 3 hospitals where the disease is common. Diagnosis was made on the basis of the clinical picture, sputum examination, and X-ray findings. Three or more specimens were obtained from each of 20 patients, 2 specimens from 34 patients, and one specimen from 20 patients.

The control group consisted of 133 serum specimens from individuals with the following diagnoses: Bacterial pneumonias (mostly pneumococcal) 22, pulmonary tuberculosis 23, febrile upper respiratory infections 17, influenza (type A), 7, coccidioidomycosis 6,† pneumonias of uncertain etiology 8, lymphogranuloma venereum 20, and normal individuals 36. All specimens in this group were taken later than the 10th day of disease and were tested while fresh.

The technic employed was as follows: Twofold serial serum dilutions commencing at

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

1 Peterson, O. L., Ham, T. H., and Finland, M., *Science*, 1943, **97**, 167.

† These specimens were supplied by Dr. C. E. Smith, Stanford Medical School.

TABLE I.
Distribution of Highest Titers Obtained by Each Individual Tested.

	Highest titer									
	<10	10	20	40	80	160	320	640	1280	2560
Primary atypical pneumonia	12	2	15	9	10	9	7	6	3	1
Control group	133	4	1*	0	0	0	0	0	0	0

* Does not include 3 specimens with titers of 20 from (1) 2 patients with pneumococcal pneumonia on third day and (2) a patient with streptococcal pharyngitis on fourth day of disease.

dilutions of 1:5 or 1:10 were made in normal saline. To 1.0 cc of diluted serum was added 1.0 cc of washed type O human cells. The mixture was thoroughly shaken and placed in the icebox at a temperature between 0° and 4°C for one hour. Coarse agglutination and accelerated sedimentation were interpreted as a positive test. Following reading, the suspensions were allowed to come to room temperature or, in the event that titers were unusually high, to 37°C in order to ascertain that the agglutination was reversible at higher temperatures. Titers were expressed in terms of the original serum dilution, *i.e.*, before addition of an equivalent volume of cell suspension.

Results in Control Group. One patient classified as having an upper respiratory infection showed a rise in titer during convalescence from less than 10 to 20. Two patients with pneumococcal pneumonia showed a titer of 20 on the third day of illness. Three patients with pulmonary tuberculosis and one with a bacterial pneumonia had titers of 10. All other sera had titers of less than 10.

On the basis of these results it seemed reasonable to consider titers of 20 to be probably significant and titers of 40 or more to be definitely beyond the normal range.

Results in Primary Atypical Pneumonia. Table I summarizes the results according to the highest titer reached by each individual tested. Sixty of the 74 patients diagnosed clinically as primary atypical pneumonia had titers of 20 or more in at least one specimen. Forty-five had titers of 40 or more. Thus approximately three-fifths had titers clearly beyond normal limits, one-fifth had titers which were probably significant, while the remaining one-fifth failed to show significant titers. It is worthy of mention that sera from 26 of those individuals who subsequently showed titers above 20 were negative when tested early in the course of the disease. Significant titers were observed more frequently in those individuals who were tested repeatedly.

The height of the titer attained showed great individual variation. Although a number of individuals tested once, and a few tested several times, failed to develop titers above 20, the majority had titers in the range between 40 and 640. The highest titer observed was 2560. The severity of the illness did not appear to have a direct relationship to the height of the titer.

It soon became clear that the time at which a specimen was taken was of considerable importance. In Table II the titers of the 130 specimens obtained from those 60 patients who at some time during illness or convalescence had titers of 20 or more are arranged according to the period of disease at which the specimen was taken. During the first week of disease less than one fifth of the sera tested showed significant titers. Rise in titer usually began between the eighth and tenth days, with the titer reaching a peak between the twelfth and twenty-fifth days and falling off fairly rapidly after the thirtieth day.

Only 2 patients with pneumonia due to psittacosis-like viruses, proven in both cases by isolation of virus from sputum, have been tested. One showed a titer of less than 10 on the eighth day of disease. Serum specimens from the other showed titers of 10, 10, 20, and 10 respectively on the sixth, tenth, fifteenth, and thirty-first days of disease. In the latter case complement-fixing antibody to lympho-

TABLE II.
Relationship of Titer to Stage of Disease of 130 Serum Specimens from 60 Patients Who at Some Time Showed Titers of 20 or More.

Titer	Day of disease specimen collected				
	Less than 7	8-10	11-20	21-30	More than 31
<20	22	4	2	2	4
>20	4	9	53	23	7

granuloma venereum antigen[2] had risen during this period from less than 6 to more than 24. Twenty-six other sera, positive by cold agglutination, failed to show a significant rise in complement-fixing antibody to the meningopneumonitis virus.[2]

Observations on Certain Properties of Cold Agglutinins. Cold agglutinins are readily adsorbable by red blood cells at low temperatures. For this reason tests are most satisfactorily done with sera separated at temperatures above 20°C. Separation of serum at low temperatures causes sufficient drop in titer to cause sera in the lower titer range (40 or less) to fall below the level considered significant. With high titer sera this is of less importance. The titer of a serum is not changed by heating at 56°C for one-half hour, but is diminished by heating at 60° and destroyed at 65°C.

Storage of serum at 4°C results in a gradual falling off in titer. Twenty specimens were retested after storage for 2 to 5 months. Ten of the 11 specimens which had shown titers of 20 or 40 when fresh had dropped to 10 or less. The 9 specimens which had shown titers in excess of 80 showed a fall in titer, but 8 of the 9 were still above 20. Two sera, stored for 10 and 14 months respectively, still showed titers of 80.

The thermal range of activity of the agglutinations varied with the titer. Two sera, with titers of 1,280 and 2,560 showed agglutination up to 21°C, but not at 25°C. This was of interest because one of these specimens had come from a patient who, after recovering from a comparatively mild primary atypical pneumonia, had died suddenly 17 days after onset as a result of pulmonary embolism.

Discussion. Progress in the field of the primary atypical pneumonias has been retarded by the fact that the etiology remains obscure. No laboratory procedure has been available by means of which the great majority of these pneumonias can be classified. In the absence of a specific etiological test the cold agglutination test may offer a roundabout way of attaining this objective. Data presented in other reports[3,4] and in this paper suggest that a large proportion of cases develop significant titers of cold agglutinins. It is not yet clear whether those cases which fail to develop cold agglutinins represent the same disease or belong in other etiological groups.

To the clinician the value of the test in the ordinary case of primary atypical pneumonia lies in the fact that it offers an objective means of confirming a diagnosis which can usually be made on the basis of the clinical picture, sputum examination, and x-ray findings The fact that cold agglutinins seldom appear in significant titer until the second week of disease means that in most instances they appear only after the patient is on the road to recovery. In certain types of cases, notably the protracted case in which diagnosis is uncertain, the rare cases of mixed "virus" and bacterial etiology, and in those in which tuberculosis or coccidioidomycosis is suspected, the test may prove especially useful.

Transmission of an infectious agent from patients with primary atypical pneumonia to cotton rats has been reported in a previous communication.[5] It is of interest that this transmission appears to be successful far

[2] Eaton, M. D., and Corey, M., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 165.

[3] Turner, J. C., Nisnewitz, S., Jackson, E. B., and Berney, R., *Lancet*, 1943, **21**, 765.

[4] Horstman, D., and Tatlock, H., *J. A. M. A.*, 1943, **122**, 369.

[5] Eaton, M. D., Meiklejohn, G., Van Herick, W., and Talbot, J. C., *Science*, 1942, **96**, 518.

more frequently in those cases which develop cold agglutinins than in those which do not. Studies on this point are in progress.

Summary. Significant titers of cold agglutinins were demonstrated in a large proportion of cases of primary atypical pneumonia. Similar titers were not observed in sera from patients with a number of other respiratory diseases.

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14358

On the Mechanism of Fever Production with Inflammation.*

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Recent studies undertaken by the writer have demonstrated the presence of a substance in exudates which offers a reasonable explanation for the basic pattern of injury in inflammation.[1,2] This substance, which is either a euglobulin or is at least associated with that fraction of exudates, has been termed "necrosin." It is presumably liberated from the cell which has been initially injured by an irritant. The internal chemistry of the damaged cell is doubtless altered yielding as a result various common denominators, which in turn are responsible for the unfolding of a fundamentally stereo-pattern in inflammation. Leukotaxine, the leukocytosis-promoting factor, and necrosin belong to such a category of chemical units formed by the injured cells.[3] In this connection necrosin has been found to induce a severe inflammatory reaction accompanied by lymphatic blockade.[1,2] This substance introduced into the circulating blood is followed by a prompt leukopenia replaced several hours later by a leukocytosis.[2] The internal organs are injured; notably the liver and to some extent the kidneys.[2] Besides fatty deposits in the parenchyma of these structures, small foci of leukocytic infiltration may be found irregularly scattered throughout these organs.[2]

The present communication summarizes further data indicating that the intravascular injection of necrosin is accompanied by a rapid elevation in temperature. This hyperthermia is not induced by other protein fractions derived from either exudate, ascitic fluid, or normal blood serum. Inasmuch as necrosin seems to penetrate from the site of inflammation into the circulating blood,[2] it is conceivable that the basis of fever production with inflammation may be referable in large part to the absorption of necrosin from the site of injury into the blood stream. Owing to limit on space the earlier literature on fever will be omitted in the present short communication.

In the present experiments dogs were injected with necrosin either in the form of an aqueous suspension or as the desiccated material which was taken up in several cubic centimeters of physiological saline. The injections were made into the circulating blood by cardiac puncture. The rectal temperature was recorded at approximate hourly intervals. The chemical preparation of necrosin from canine exudates has been described in earlier

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1 Menkin, Valy, *Science*, 1943, **97**, 165.

2 *Idem, Arch. Path.*, 1943, **36**, 269.

3 *Idem, Dynamics of Inflammation*, Macmillan Company, 1940.