ing appropriately to at least 12 specific stimulus patterns presented in successive fashion. In further studies, it would be possible to determine the maximum number of specific patterns to which these animals could respond effectively. Until such studies have been completed, investigations of complex discriminations with chimpanzees should be interpreted with caution, until the bases of the discriminations have been assessed.

With the procedure described above, concept formation was a function of the concept problem. The common element of the first concept problem (the bottom row of windows) had a specific spatial location; the common element of the second concept problem (any three windows) did not. This difference in the level of abstractness of the two problems may have been an important factor. With a different procedure, the chimpanzees could probably have been trained to respond to the common element of the second concept problem. If the stimulus patterns were changed after each sequence without changing the concept, for example, the animals would have been unable to maintain a discrimination by responding to specific patterns.

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11 June 1958

Interpretation of Properdin Levels Determined by Phage Neutralization Technique

It has been demonstrated by Van Vunakis, Barlow, and Levine (*1, 2*) that the properdin system neutralizes the *Escherichia coli* phage T2r$^+$ (*3*). These authors have described a precise and reproducible procedure for determining the phage neutralizing (PhN) activity of human serum and have suggested that it affords a means of measuring properdin levels (*4*). In brief, the technique measures phage neutralizing activity in terms of the amount of serum (PhN_{50}) required to neutralize 50 percent of the phage introduced into the system under the experimental conditions used. During the course of an investigation of the influence of complement (C′) on the neutralization of bacteriophage by immune antibody, some observations have been made that indicate the necessity for caution in interpreting properdin titers determined by the phage procedure. In addition, these findings suggest that concepts concerning the nature of the properdin system should be carefully reconsidered.

Table 1. Neutralization titers of human sera tested with T2, T6, and T7 bacteriophage. Diluent: 0.126*M* Veronal buffer, *p*H 7.4 to 7.6, containing 0.1 percent 5X crystalline bovine serum albumin (Armour & Co.), Ca^{++} (0.00015*M*), and Mg^{++} (0.0005*M*). Procedure: Reaction mixture tubes, sitting in an ice bath, were prepared, diluent (to give a final volume of 1.5 ml), serum dilutions, and phage (0.5 ml containing 7.5×10^4 infectious units) being added in that order. The tubes were well mixed and incubated for 90 minutes at 37°C. They were then chilled, diluted, and titrated for residual phage. PhN_{50} titers were calculated as described by Barlow *et al.* (*4*).

Serum	PhN_{50}/ml and indicator phages*			Hemolytic titer ($C'H_{50}$/ml)
	T2	T6	T7	
NH	20	24	19	24
B	15	20	28	Not done
KC	12(12)	28(24)	42(40)	31
G	25(22)	19(16)	7(8)	31
DM	24(24)	24(21)	56(43)	30
SB	16(14)	27(27)	4(3)	32
AC	9(8)	26(25)	13(10)	28
DB	15	31	35	41

* Figures in parenthesis are serum titers determined 3 to 4 months after the initial assay. Sera were stored at −35°C.

Several different phages of the T series (T2, T6, and T7) were to be used in this study, but the presence of normally occurring neutralizing substance (properdin) in the guinea pig serum being used as a C′ source presented an obvious complication. As was indicated by Barlow *et al.* (*2*), repeated absorptions of the serum with zymosan resulted in a C′ reagent essentially devoid of phage neutralizing activity. The present report stems from the observation that, prior to absorption with zymosan, this serum neutralized these three phages to varying degrees. It was wondered whether this variation could be attributed to a difference in susceptibility of the phages, or whether it perhaps indicated that this serum contained varying amounts of neutralizing substances directed against the different phage types. It was felt that these questions could possibly be answered by assaying several sera and by using all three phage types. If the differences observed were due to varying susceptibility of the phages to a single neutralizing substance, one would expect the ratio of serum titers obtained with the three different phages to remain approximately constant from one serum to another. On the other hand, the failure to obtain such a correlation would suggest that the neutralizing substance(s) had some degree of specificity and could be present in different sera in varying amounts.

Several human sera showing normal hemolytic C′ levels were assayed for activity against T2, T6, and T7 phages by a procedure similar to that described by Barlow, Van Vunakis, and Levine (*4, 5*). Heated serum samples were included in all cases, and no significant neutralization was found. As is shown in Table 1, no constant ratio occurs—that is, the phage neutralizing activity of a serum against one of the phage types does not necessarily correlate with its activity toward the other two. It is clear that if one were to use the neutralization of T6 or T7 as an indicator of serum properdin levels, one might obtain a distinctly different impression than he would if T2 were used. It is obvious that circumspect judgment is necessary in the evaluation of properdin levels determined by this procedure, and indeed, the question of the true nature of properdin is raised.

These findings not only raise the question of the significance of properdin levels determined by the phage technique but also indicate that properdin may have some degree of specificity which is suggestive of antibody. Nelson (*6*) has presented evidence and a hypothesis suggesting that the properdin system may be explained in terms of normally occurring antibody operating in conjunction with C′ without introducing the concept of the new entity, properdin. Skarnes and Watson (*7*) have indicated that properdin and normal antibody may be the same substance in view of the numerous properties shared by them.

From the data given in the present report, and also from the failure of others to find a satisfactory correlation between properdin titers determined by the zymosan assay method (*8*) with those determined by the phage tech-

nique (*4*) or the tanned erythrocyte procedure (*9*), it is suggested that these discrepancies may be attributable to the fact that different substances are being measured by the different indicator systems. Thus, one is drawn to the conclusion that either there are several different properdins, or that properdin is a family of cross-reacting antibodies capable of combining with zymosan. The inability to correlate properdin levels determined by the various procedures might then be due to the fact that antigens such as tanned erythrocytes, T2, T6, and T7 phage are measuring a specific portion of the properdin or normal antibody pool which may vary both in quantity and quality from serum to serum.

Before the conclusion is drawn that properdin is normal antibody, however, one distinguishing feature of the properdin system should be stressed. This is the demonstration by Pillemer *et al.* (*10*) that C′ is required not only for the manifestation of the viricidal, bactericidal, and hemolytic reactions attributed to the properdin system but also for the combination of properdin with zymosan and other polysaccharides reactive in the system. While C′ may be essential for bactericidal and hemolytic reactions by immune antibody, it is clear that it is not required for the formation of antigen-antibody complexes. Thus, if properdin is to be considered normal antibody, it will be necessary to revise our concepts concerning the nature of normal antibody-antigen reactions in order to incorporate C′ as an essential cofactor. While it may be premature to postulate a role for C′ in such reactions, the multivalent or cross-linking concept of C′ as presented by Weigle and Maurer (*11*) might be considered with respect to this problem. It is possible to envisage C′ as a stabilizer of readily dissociable immune complexes by virtue of its cross-linking activity. Thus, it might both play a role in the formation of certain low-avidity antibody-antigen complexes and serve as an essential component for the manifestation of such unions where bactericidal or hemolytic reactions are involved. It is hoped that experiments being undertaken may shed further light on the role of C′ in properdin or normal antibody reactions, or both (*12*).

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3. Reference 1 cites a personal communication from Ralph Wedgwood indicating that T7 is neutralized in the same manner as T2.
4. J. L. Barlow, H. Van Vunakis, L. Levine, *J. Immunol.* 80, 349 (1958).
5. One deviation involved the use of a diluent for the reaction mixtures different than that used by Barlow *et al.* (*2*). The Veronal buffer used in the assays reported in Table 1 is the same as that described by M. M. Mayer *et al.* [*J. Exptl. Med.* 88, 427 (1948)] except that the NaCl concentration was decreased so that the final molarity of the buffer was 0.126. This buffer contains bovine serum albumin, Ca^{++} (0.00015*M*), and Mg^{++} (0.0005*M*). Control experiments performed on three sera (SB, KC, and AC) indicate that the buffering system or Mg^{++} level used by Barlow *et al.* (*2*) gives essentially the same titers and patterns presented in Table 1 for these sera.
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12. I should like to acknowledge the advice and encouragement given by Dr. Robert A. Nelson, Jr., during the course of this work. The generous supply of various reagents, prepublication information, and constructive criticism from Mr. J. L. Barlow, Dr. H. Van Vunakis, and Dr. L. Levine is gratefully acknowledged.

8 April 1958

Infection of Human Volunteers with Type 2 Hemadsorption Virus

The hemadsorption viruses, types 1 and 2, are new members of the myxovirus group which have recently been recovered from infants and children with respiratory illness (*1*). Subsequent studies have provided evidence that a considerable proportion of respiratory illness in children during the winter of 1957–1958 was associated with the hemadsorption viruses (*2*). A study to determine whether type 2 virus could produce infection and illness in adults was performed with male volunteers (*3*). Although placebo controls were not employed in this pilot study, valuable information was acquired because illness was correlated with the occurrence of infection.

The virus used for the inoculum was derived from an infant with acute laryngotracheobronchitis. The original throat swab specimen from this infant was inoculated into a monkey kidney tissue-culture flask maintained with Eagle's basal medium without serum. After 7 days' incubation at 36°C the tissue culture fluid was filtered through a Selas 0.03 filter and stored at −50°C. Safety tests were carried out in monkey kidney and HeLa cultures and in suckling and adult mice, guinea pigs, rabbits, and various bacteriologic media. No bacteria or contaminating viruses were detected.

Thirty-two healthy male volunteers, ranging in age from 21 to 46 years (mean, 28 years) were selected from among the inmates at the Patuxent Institution at Jessup, Md. Seven of the men did not have neutralizing antibody for type 2 virus. The remaining 25 volunteers had antibody levels of 1:4 to 1:128 (mean of 1:21). The volunteers were isolated in separate cells and had an independent medical examination each day by two physicians. The laboratory techniques for virus isolation, virus identification, and serologic testing by hemagglutination-inhibition, complement-fixation, and neutralization have been described (*1*).

Each volunteer was given a total of 1 ml of undiluted tissue culture fluid by swabbing the posterior oropharynx and the lower palpebral conjunctivae and by spraying and instillation into the nose and oropharynx. Each volunteer received 80 TCD_{50} as determined by a simultaneous titration.

Throat swabs taken from all the volunteers on the 2nd, 3rd, 6th, and 7th days were tested simultaneously for virus in monkey kidney tissue culture by the hemadsorption technique (*4*). Type 2 virus was recovered from 24 of the men. Virus was not detectable on the 2nd day after inoculation but was isolated from 10 of the volunteers on the 3rd day. Twenty-two were positive on the 6th day, with 12 of the isolations coming from individuals with a negative test on the 3rd day. Nineteen volunteers had virus on the 7th day, with two of these individuals shedding virus for the first time.

A total of 25 men developed a rise in antibody for type 2 virus. Each of the volunteers from whom virus was recovered had a serologic response. Twenty-two men developed a rise in neutralizing antibody, 18 in hemagglutination-inhibition antibody, and 14 in complement-fixing antibody. The one individual with a positive serologic response from whom virus was not recovered developed antibody demonstrable by neutralization, hemagglutination-inhibition, and complement-fixation. None of the volunteers developed a rise in complement-fixing antibody for adenovirus or Asian influenza.

When clinical illness failed to develop by the 5th day, the men were released from isolation. On the 6th day, however, six of the men reported to the infirmary complaining of respiratory illness and immediately all volunteers were returned to isolation. Examination of the other volunteers revealed that additional individuals had onsets of respiratory illness on this day. The onset of these illnesses coincided with the time when the greatest number of volunteers had virus demonstrable in their upper respiratory tracts. For the most part the illness was mild with prompt and uneventful recov-