but less striking, percentage changes when pyrogen-free saline was injected. However, the insertion through the abdominal wall of a sterile syringe needle alone gave rise to changes as great as 44 percent. Finally, we found that the cardiac bleeding procedure itself led to "relatively large" percentage changes. Consequently, we designed an experiment to confirm the last observations.

Four-week-old male, albino, guinea pigs were obtained (*2*) and maintained on Purina Guinea Pig Chow, alfalfa, and water. Lettuce, as a source of vitamin C, was given several times daily. After 1 month the animals were bled by cardiac puncture. Exactly 24 hours later the guinea pigs were bled again. The number of punctures (number of times the chest was entered) and the number of bleedings required to obtain 1 to 1.5 ml of blood were recorded per animal. The serums were stored at −60°C until titrated. All assays for complement (*3*) were performed on the same day with the same reagents, the method being modified by using (i) veronal-buffered saline containing 0.0005*M* Mg^{++} and 0.00015*M* Ca^{++} as diluent, (ii) sheep erythrocytes preserved in modified Alsever's solution, and (iii) a 45-minute incubation period at 32°C. For guinea pig complement, hemolysis is greater at 32°C than at 37°C and the reaction is essentially complete after 45 minutes (*4*). The titers were estimated from the logarithmic form of the von Krogh equation (*5*) by the method of least squares, and not by the graphic "free-hand" method. The results are presented in Table 1.

The percentage changes in complement titers ranged from approximately −50 to 15. Nine of the changes were negative and two were positive. The loss of blood was roughly 5 to 8 percent of an animal's total blood volume.

We estimated our experimental error by repeatedly testing, simultaneously, a pool of guinea pig serum. Five tubes were assayed against sheep erythrocytes which had been preserved in modified Alsever's solution (A) and five against erythrocytes from defibrinated sheep blood (D). The coefficients of variation were 2.3 percent for A and 5.3 percent for D.

The mean of the differences between the pairs T_1 and T_2 of each animal is significantly different from zero ($t = -2.9574$; $.01<p<.02$). This significant difference is the basis for our statement that an individual's complement activity may not be relatively stable.

Because of the small size of the sample we attach little importance to the significant ($p = .02$) correlation coefficient for (i) T_1 and T_2 and (ii) T_1 and body weight at 0 hours.

We have been unable, as yet, to uncover any reason for the variations between the T_1 and T_2 values. We assumed that the unassessable stresses arising from the restraint of the animals during the cardiac bleeding procedure and the stresses connected with the process of collecting the blood itself were contributory. However, we failed to demonstrate, by ranking methods, any correlation between (i) T_1 and P_1 (number of punctures at 0 hours), (ii) T_1 and B_1 (number of bleedings at 0 hours), (iii) T_2 and P_2 (number of punctures at 24 hours), (iv) T_2 and B_2 (number of bleedings at 24 hours), (v) T_2 and $P_1 + P_2$, and (vi) T_2 and $B_1 + B_2$.

We began to test the ideas that neurologic responses or humoral factors or both come into play during and between the bleeding procedures. We cannot conclude from preliminary experiments that etherization of the animals prior to bleeding lessened the response or, in other experiments, that the intraperitoneal injection of histamine accentuated the response.

In view of the phenomenon described we believe that the inferences based on changes in complement reported for various human diseases, infectious or otherwise, must be reviewed. Currently, hypotheses are being formulated suggesting that autoimmune mechanisms may possibly play an etiologic role in certain diseases (*6*). The changes in complement activity are reported to be related to exacerbation and remission of the disease in question. We are not yet convinced that changes in complement values necessarily reflect changes in a disease process. Until proved otherwise we must consider the possibility that such changes in titer are not unusual.

We believe that many of the shortcomings in the studies of complement in vivo arise because attention has not always been paid to experimental design, to statistical analysis of the results, to the determination of complement titers by procedures more precise than the graphic "free-hand" method, and to attempts to compare titers obtained on different days employing different sheep erythrocytes. The validity of the last practice is open to question in the absence of experimental evidence that different batches of sheep erythrocytes react similarly.

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14 May 1964

Poliovirus: Growth in Non-Nucleate Cytoplasm

Abstract. *Cytoplasmic fragments were produced by micromanipulation of cells from a human amnion cell line cultured on coverslips. The cultures were infected with type 1 (Mahoney) poliovirus, and incubated for 7 hours with tritiated uridine (H^3U). Fluorescent antibody to the poliovirus indicated antigenic sites in a number of non-nucleate fragments. By autoradiography the incorporation of H^3U was demonstrated at some of the same sites. The occurrence of poliovirus antigen at the same site as induced synthesis of RNA in non-nucleate cytoplasm of mammalian cells indicates that poliovirus infection and growth occurred independently of immediate contribution from the nucleus.*

Certain picornaviruses have been shown to develop in cells in which nuclear RNA synthesis was inhibited by treatment with actinomycin D prior to infection (*1, 2*). Even without actinomycin D, the infectious process was accompanied by suppression of nuclear RNA synthesis (*2*), indicating a virus-induced inhibition of this nuclear function. While growth of these picornaviruses occurs under conditions which stop normal synthesis of nuclear RNA,

Table 1. Location of viral antigens indicated by fluorescent antibody (Ab) and autoradiographic detection of RNA synthesis after infection of non-nucleate cytoplasmic fragments with type 1 poliovirus.

Expt. No.	Non-nucleate cytoplasmic fragments							Infected intact cells (%)
	Total number observed	Presence of Ab		Labeling of RNA				
				In Ab+ fragments		In Ab− fragments		
		+	−	RNA+	RNA−	RNA+	RNA−	
1	5*	1	4					40
2	7*	3	4					40
3	7	6	1	3	3		1	77
4	3	2	1	2			1	
5	2	2	0	1	1			
6	4	1	3	1			3	75
Totals	28	15	13	7	4	0	5	

* Only antigen was examined in these experiments; labeling was not done.

this evidence does not establish whether or not the infectious process is dependent on the nucleus. The direct test of the hypothesis that picornaviruses require only cytoplasmic resources must be made in viable cytoplasm, physically free of a nucleus.

Non-nucleate cytoplasmic fragments from a human amnion cell line have been prepared by micromanipulation and have been found to be motile, to undertake pinocytosis, and to synthesize protein but not RNA. Fragments remain spread on glass until an abrupt shrinking and "bubbling" activity appears as viability is lost (*3*). The survival of these fragments is associated with declining competence for incorporating amino acids (*4*), and is presumably dependent upon residual and functional RNA previously delivered from the nucleus. Such fragments are capable of being infected and of sustaining at least the first 20 hours of development of an ornithosis agent, with morphologic maturation and synthesis of DNA of the ornithosis agent (*5*).

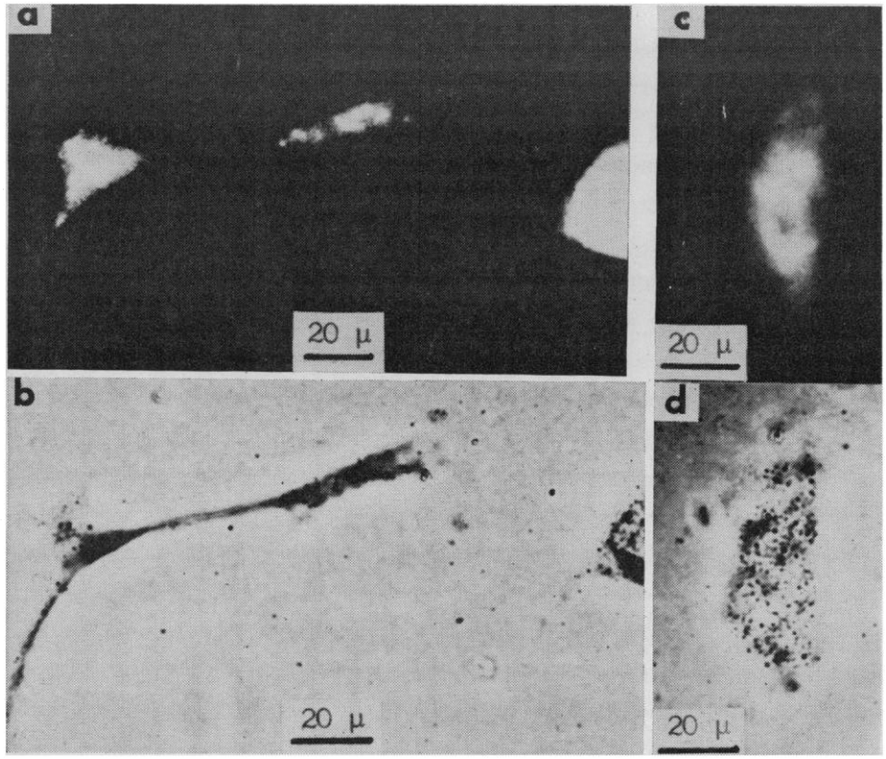

Fig. 1. Non-nucleate cytoplasmic fragments of amnion cells infected with type 1 (Mahoney) poliovirus and fixed for 5 hours after a 2-hour initial period of contact with virus. (*a*) One non-nucleate fragment showing foci of fluorescent antibody localization and a portion of an intact cell with generalized distribution of antigen. (*b*) Same field as (*a*), showing less intense labeling with H^3U in the non-nucleate fragment and the adjacent intact whole cell. The label is more concentrated over areas in which antigen was detected. (*c*) A non-nucleate fragment containing clustered sites of antibody localization. (*d*) The same field as (*c*) showing moderately intense labeling with H^3U.

One to ten non-nucleate fragments were prepared in Rose chambers as described previously (*5*), about 10^4 cells of amnion cell line 185 (*6*) being used in a medium consisting of Medium 199 (*7*), Hanks solution, and human serum (45:45:10, by volume). A stock of type 1 (Mahoney) poliovirus containing 2×10^8 plaque-forming units of virus per milliliter was prepared in human amnion (FL) cell cultures maintained after infection in a serum-free, yeast extract-lactalbumin hydrolysate medium. This preparation was diluted with an equal volume of a serum-free solution consisting of Medium 199 and Hanks solution (1 : 1), containing 10.0 μc of H^3U per milliliter (*8*). After two washes to remove the serum-containing medium, 0.8 ml of the labeled, diluted virus preparation was added per chamber. After 2 hours' incubation, the inoculum was replaced with medium consisting of Medium 199, Hanks solution, human serum (40:40:20, by volume) and 5 μc of H^3U per milliliter; incubation was continued for 5 hours. The non-nucleate fragments and intact cells, still attached to the coverslips, were washed twice with warm medium, air dried, extracted for 10 minutes in cold acetone, dried again, and stored at 4°C.

Antiserum to type 1 poliovirus was produced by immunizing a monkey with virus from monkey kidney cell cultures. The antigen consisted of equal parts of infectious culture fluids and adjuvant (9 parts of white mineral oil and 1 part of Arlacel A). This serum was labeled with fluorescein and passed through Sephadex (*9*). The labeled serum was diluted 1 : 20 and applied over the area of the cells which were then placed in a moist chamber for 30 minutes. Antibody was washed off with two changes of 0.15*M* saline buffered with 0.01*M* Na_2HPO_4 and each coverslip mounted, cells down, with 1 : 10 buffer in glycerol. The non-nucleate fragments and adjacent intact cells were viewed and photographed on color film, dark field optics being used with ultraviolet illumination. Control, noninfected cultures were similarly stained and examined.

The coverslips were then washed in distilled water, immersed for 1 hour in cold, 5-percent trichloroacetic acid, rinsed in four changes of distilled water,

air dried, mounted face-up on microscope slides, and covered with AR-10 stripping film (*10*). After development, photographs were made by phase-contrast microscopy to correlate areas identified by bound antibody with areas of localization of H^3U incorporation.

Of 28 non-nucleate fragments surviving exposure to virus inoculum and subsequent manipulation, 15 bore foci of antigen (Table 1). Of 16 studied by both fluorescent antibody and autoradiographic methods, 11 contained antigen and of these, 7 also showed H^3U incorporation at the same sites (Fig. 1). All of the intact cells which contained antigen incorporated H^3U into cytoplasmic foci of virus growth.

In some experiments, non-nucleate cytoplasmic fragments bore clear-cut foci of antigen but appeared to be rather lightly labeled with H^3U (Fig. 1, *a* and *b*). This was probably due to the autoradiographs being underexposed, since such preparations also showed a minimum amount of labeled cytoplasm in clearly infected intact cells.

In some experiments yielding good autoradiographs, most non-nucleate fragments in which fluorescent antibody fixation occurred were also labeled at the same sites (Fig. 1, *c* and *d*). Those non-nucleate fragments which acquired no antigen were presumed to be uninfected just as some intact cells were, by the same evidence, not infected.

Foci of antigen formation were usually discrete in non-nucleate fragments but were diffuse in the intact cells of the same cultures. The discrete sites in non-nucleate fragments could represent regions in which new virus was synthesized more slowly than in intact cells. Infected cultures containing only intact cells were therefore examined with fluorescent antibody at 0, 2, 3, 4, and 5 hours after the initial period of contact with inoculum. No cells bore foci of antigen in less than 3 hours, but at 3 and 4 hours, many infected cells contained discrete cytoplasmic foci of antigen. These foci and the foci found at 5 hours in non-nucleate cytoplasmic fragments were morphologically alike in shape, size, and intensity of "staining" with fluorescent antibody. It is concluded that synthesis of new viral antigen occurred at a somewhat slower rate in non-nucleate cytoplasmic fragments than in whole cells.

The concurrence of poliovirus antigen and tritiated uridine incorporation in non-nucleate cytoplasmic fragments, under conditions producing results comparable with the process in intact cells, indicates that the poliovirus induced both specific antigen formation and new RNA synthesis in the non-nucleate cytoplasmic fragments and that the cell nucleus did not participate in this sequence of steps in the infectious process.

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13 April 1964

Allotypic Specificities of A- and B-Chains of Rabbit Gamma Globulin

Abstract. *The extent to which the A-chain of γ-globulin reacted with antibody against an allotypic specificity controlled by the* b *locus was about that expected from the contamination of A-chain with B-chain; the* a *locus specificity was confined only to A-chain. Thus, data were consistent with complete separation of allotypic specificities,* a *locus with A-chain and* b *locus with B-chain.*

Allotypic specificities (*1*) of rabbit γ-globulin (that is, antigenic specificities each of which is found in some but not in all rabbits) were shown to fall into two genetic groups, *a*, having specificities Aa1, Aa2, Aa3, or *b*, having specificities Ab4, Ab5, Ab6 (*2*)—by the nomenclature of Dray *et al.* (*3*); loci *a* and *b* are not closely linked (*4*). It was observed in 1961 by Oudin (*5*) and confirmed since then (*6*) that specificities of each group *a* and *b* may be carried by a single γ-globulin molecule.

A model of rabbit γ-globulin has been proposed (*7*, *8*) containing two pairs of peptide chains, A and B in the nomenclature of Porter (*7*), which will be followed here or, respectively, H and L in the nomenclature of Edelman and Benacerraf (*9*). Specificities of the *a* and *b* groups have been looked for in A- and B-globulin fractions with the result that B-fraction had only *b* specificities, while A-fraction had specificities of both *a* and *b* groups (*10*). A later report (*11*) suggested that either *b* specificities were actually carried by both chains, or the amount of contamination of the A-fraction would have to be "remarkably great" to account for the heavy precipitate obtained when tested with antiserum of the *b* group. The present report concerns the quantitative measurement of the capacity of the A preparation to combine with antibody against a specificity of the *b* group (Ab4), with the object of finding if this capacity may be explained by a contamination. If rabbit allotypic specificities are due to genetically controlled primary structure variations, one would expect each type of chain to carry only one of the two unlinked locus specificities, as seems the case for human Gm and Inv (hereditary globulin factors) specificities (*12*).

A pool of rabbit serums with Aa1 and Ab4 specificities was precipitated with 1.75*M* ammonium sulfate, and the precipitate was dialyzed against phosphate buffer (0.025*M*, *p*H8) and passed through a diethylaminoethyl (DEAE) cellulose column. The effluent was concentrated by precipitation with 2*M* ammonium sulfate and the precipitate was dialyzed against 1*M*, *p*H8 tris buffer. Only γ-globulin (RGG) was found by starch-gel electrophoresis (*13*). The A- and B-chains were prepared according to Fleischman *et al.* (*14*) by reduction of γ-globulin with 0.2*M* mercaptoethanol followed by *S*-