Table 2. Influenza serum (human) before and after absorption by column antigens.

Column antigen	Serum CF titer with antigens*		Serum HAI titer with antigens†	
	A'/ Hawaii/ 303/56	A2/ Jap/ 305/57	A'	A2
None	80	80	80	10
A'/Hawaii/ 303/56	<10	<10	<5	<5
A2/Jap/ 305/57	<10	<10	80	<5

* Reciprocal of the highest dilution of serum-fixing complement in the presence of four units of antigen.
† Reciprocal of the highest dilution of serum inhibiting four hemagglutinating units of virus.

ment of flow in columns. The usual cationic character of serum antibodies at neutral *p*H contrasts with the anionic nature of many proteins at this *p*H. The anion-exchange capacity of ECTEOLA cellulose (*2*) was employed in the study reported here, to allow attachment of several antigens but not attachment of antibodies.

Antigens were prepared from three groups of organisms: adenoviruses from frozen and thawed infected HeLa cells and tissue-culture fluid; influenza virus from ether-treated (*3*), infected raw chick-embryo allantoic fluid; and typhus group rickettsiae from infected yolk sacs, ether-treated by method No. 3 of Topping and Shepard (*4*). These antigens were placed on columns of ECTEOLA cellulose, capacity about 0.3 meq/g, in 0.005*M* potassium phosphate buffer (*p*H 7.2), at room temperature, by the method of Hoyer *et al.* (*5*). Complement fixation (CF) tests with appropriate guinea pig or human antisera on each effluent indicated no antigen—that is, full antigen adsorption. Furthermore, there was no hemagglutinating antigen in the effluents from columns exposed to influenza antigens. After the column had been washed with the same buffer, an aliquot of human or guinea pig antiserum, dialyzed to the same conditions, was passed over the antigen-loaded column. The effluent was concentrated to the original serum volume by dialysis against polyethylene glycol (*6*) and assayed for antibody content with standard complement-fixation, neutralization (NT), or hemagglutination-inhibition (HAI) procedures. For complement-fixation and hemagglutination-inhibition tests, antigens were prepared as described above. For the typhus neutralization determinations, yolk-sac suspensions of viable *Rickettsia prowazeki* and *R. mooseri* were employed.

Table 3. Epidemic typhus serum (guinea pig, convalescent) before and after absorption by column antigens.

Column antigen	Serum CF titer with antigens*		Serum NT titer with toxic suspensions†	
	Epidemic	Murine	Epidemic	Murine
None	160	40	128	8
Epidemic	<10	<10	8	4
Murine	80	<10	112	<2.5

* Reciprocal of the highest dilution of serum-fixing complement in the presence of four units of antigen.
† Reciprocal of theoretical dilution of serum protecting two of four mice from 2 LD_{50} of toxic suspension, interpolated by the method of Reed and Muench.

Typical results are shown in Tables 1–3. It may be noted that adenovirus complement-fixation antibody to three types was removed by either a homologous or a heterologous type antigen (Table 1). Similarly effective was the absorption of complement-fixation antibody from a human serum by two type-A influenza strains (Table 2). Hemagglutination-inhibition antibodies were removed more selectively by the A2 than by the A' antigen, but the low initial A2–hemagglutination-inhibition titer makes interpretation difficult. Epidemic typhus antiserum lost essentially all complement-fixation and neutralization antibody after homologous absorption, while heterologous absorption reduced predominantly the heterologous antibody (Table 3).

Certain additional reciprocal cross-absorption experiments have been performed, with similar results. The typhus system has been the most fully studied. Characterization of the type-specific adenovirus antibodies was not possible with the sera studied because, following the preabsorption dialysis, no type-specific antibody was detectable in these sera. The specificity of absorption of influenza hemagglutination-inhibition antibody requires further study. Storage of certain antigen-loaded columns, at 4°C, between repeated absorptions for periods up to 2 months did not impair their absorbing ability. This observation suggests that Formalin treatment of antigens (*7*) may allow not only additional useful storage but also column adsorption of other antigens with less initial affinity for anion-exchange materials.

The most obvious interpretation of these data seems to be that antibody combined with column-bound antigen which remained *in situ*. The possibility that antibody, once united with antigen, eluted in a serologically inactive form cannot be excluded, particularly from the hemagglutination-inhibition and neutralization data. However, antigen-antibody complexes in serum usually fix complement alone and are thus discerned as "anticomplementary sera." Anticomplementary sera were not encountered with this system except when DEAE cellulose (*2*), of 0.93 meq/g capacity, was employed under identical conditions.

This absorption method, in which soluble antigens attached to cationic particulates are used, may provide a sensitive tool for the differentiation of virus strains and for quantitative studies of antibody response after infection. More diverse application of this methodology to other antigen-antibody systems is suggested by the considerable number of proteins and microorganisms adsorbable to the cellulose anion exchangers.

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A "D"-like Antigen in Rhesus Red Blood Cells and in Rh-Positive and Rh-Negative Red Cells

Abstract. A "D"-like antigen has been demonstrated in human and rhesus red cells. These red cells, as well as heat extracts of human blood (Rh-positive or Rh-negative), induce formation of "D"-like antibodies in guinea pigs. These antibodies, when exposed to rhesus red cells or to Rh-positive or Rh-negative red cells, yield eluates of "D"-like specificity.

The unexpected findings of Murray (*1*) and Murray and Clark (*2*) that heat extracts of Rh-positive or -negative red cells, when injected into guinea pigs, produce an antibody which they believe showed anti-D specificity have been confirmed. Red cells washed three times were suspended in an equal volume of saline and heated at 50°C for 20 minutes. The red cells were removed by centrifugation at 1500 *g* for 10 minutes. Guinea pigs were injected intraperitoneally twice with 5 ml of the heated extracts at an interval of 3 days and tested 10 days later.

In two different series we injected 12 guinea pigs with extracts of D--/D-- red cells containing an excess of D

Table 1. Ready production of the "D"-like antibody.

Injection	Number	
	"D"-like antibody produced	No antigenic response
Heat extracts of Rh-negative red blood cells (rbc)	13	7
Heat extracts of Rh-positive (R_2r) rbc	4	4
Heat extracts of Rh-positive (D-- /D--) rbc	6	6
Rh-positive (R_2R_2) rbc	7	2
Washed sediments from heat extracts of Rh-positive rbc	6	4
Washed sediments from heat extracts of Rh-negative rbc	5	5

antigen, and 6 out of 12 produced anti-D-like specificity, but only one gave strong reactions. Of the 20 animals injected with heat extracts of Rh-negative blood, 13 produced good to moderate effects. In all cases the sera were absorbed with Rh-negative blood in dilutions of 1:5 or 1:10 and tested with red cells suspended in saline.

Ponder and Ponder (*3*) had shown that the heat extracts when ultracentrifuged yielded a sediment consisting of tiny fragments and "myelin" forms released from the red cells by heating. These were readily visible by phase microscopy. Subsequently, Murray and Clark (*2*) demonstrated that guinea pigs, when injected with the washed sediment derived from heat extracts of Rh-negative blood and centrifuged at 10,000 rev/min, produced an antibody which they believed showed anti-D specificity. This observation was confirmed in our experiments with the heat extracts of Rh-positive blood, sedimented at 30,000 *g* and washed three times at the same or higher centrifugal fields. Of ten animals, each injected twice with the sediment derived from the heat extracts of a total of 20 ml of whole blood, six produced good antibody response. In similar experiments with the sedimented particles from heat extracts of Rh-negative blood, five out of ten guinea pigs produced the antibody.

The production in guinea pigs of an antibody with "D"-like specificity upon injection of Rh-positive, Rh-negative, or rhesus red cells was demonstrated.

Table 1 records the results of injecting (*4*) guinea pigs with heat extracts (50°C) of Rh-negative red cells, heat extracts of two series of Rh-positive red cells, one series with Rh-positive red blood cells (R_2R_2), the washed sediment from heat extracts of Rh-positive blood, and also from Rh-negative blood. In each of these series there is a high incidence of production of the "D"-like antibody.

The relation of the "D"-like antigen in Rh-positive and Rh-negative red cells or their extracts and in rhesus red cells to the usual D antigen of Rh-positive blood requires extensive study. Thus far, the "D"-like antibodies produced in guinea pigs seem to differ from human anti-D in several respects, among which the following are cited: (i) Rh-positive red cells blocked with a powerful blocking anti-D [or selected cells sensitized with anti-c (hr′)] are still agglutinated by the "D"-like antibody produced in guinea pigs injected with rhesus red cells or heat extracts of Rh-positive or Rh-negative blood. (ii) The "D"-like antibodies induced by any material containing the "D"-like antigen when exposed to Rh-positive, Rh-negative, or rhesus red cells yield eluates showing "D"-like specificity, while Rh-negative red cells, possessing as they do the "D"-like antigen, when exposed to human anti-D do not yield active eluates.

No antibody to the "D"-like antigen is obtained if the heat extracts are prepared from either trypsinized Rh-positive or Rh-negative red cells (*5*).

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Effect of Strychnine upon the Electrical Activity of an Isolated Nerve Cell

Abstract. The effect of strychnine upon the electrical activity recorded from the axon and the soma of an isolated nerve cell (the nonadapting stretch receptor cell of the crayfish) was studied. Protracted exposure of the soma of the cell to strychnine prolongs the duration of the intracellulary recorded action potential, as has been described in other excitable tissues treated with quaternary ammonium ions. During the plateau in the falling phase of the soma spike, the axon is usually firing repetitively. The stimulation of the inhibitory fiber produces a premature termination of the prolonged spike.

The effects of strychnine upon the activity of a single nerve fiber are known (*1*). Strychnine has also been extensively used in electrophysiological investigations of central nervous system activities. In these experiments, however, the inferences which can be drawn concerning the actions of the compound are limited because of the complex structural and functional arrangements in the central nervous system. We have not seen in the literature a study of the effect of strychnine upon the electrical activity of a single, isolated nerve cell.

The stretch receptor of Crustacea (*2*), which has been studied physiologically by several investigators (*3, 4*), is a suitable preparation for such a study. The soma of the isolated nerve cell can be easily penetrated by microelectrodes, and both excitatory (the generator potential set up by stretch) and inhibitory stimulation may be applied.

Nonadapting abdominal stretch receptors of crayfish were dissected and mounted on a device similar to that described by Eyzaguirre and Kuffler (*4*). The fast-adapting receptor was cut off. The preparation was suspended in a plastic box containing Van Harreveld solution. The axon was raised into a layer of mineral oil where platinum electrodes recorded the discharge. Micropipets filled with potassium chloride were used to penetrate the cell; they were mounted in a bridge circuit permitting simultaneous recording and stimulation. After the addition of a strychnine sulfate solution (1 percent) to the perfusion fluid (final concentration about 0.03 percent), the exceedingly regular discharge of the receptor to a steady stretch was broken and bursts of spikes were recorded from the axon, which were followed by pauses.

The cell must be depolarized, either by stretch or by application of a pulse of cathodal current through the impaling microelectrode, for the strychnine type of activity to appear. The threshold to direct stimulation was not altered appreciably, but a short burst of spikes (two or three) was initiated at a rheobasic current. The membrane resistance and the resting potential were not altered. The frequency of occurrence of the bursts increased with increasing degree of stretch, while the number of spikes in the bursts decreased. A total desynchronization with a return to the regular discharge of the receptor could be obtained if a high degree of stretch was applied or if calcium ions were added to the solution.

The changes described are due to the action exerted by strychnine mostly upon the membrane of the cell (and possibly the initial portion of the axon) rather than upon the axonal membrane (at the concentration used). When the muscle bundle and the soma of the cell were kept in oil, with only several millimeters of the axon being lowered in