Table 1. Mean pain thresholds (in milliamperes).

Test No.	Sensory deprivation group	Control group
1	0.290	0.240
2	0.182	0.221
Difference	0.108	0.019

be considered reliable. The criterion of reliability was taken to be variability of less than 10 percent for an entire practice period. If a subject did not meet this criterion in the course of four practice periods he was eliminated from the study. The practice periods were discontinued at the conclusion of the period in which the criterion was met.

The sensation of pain was elicited by applying an electrical current of a frequency of 1000 cy/sec through small dry electrodes clamped to either side of the lobe of the right ear. The electrodes were brass disks, 7 mm in diameter, which were firmly clamped to the ear lobe but caused no discomfort. The weight of the electrodes, of the clamping device, and of the connecting wires was supported in such a manner that there was no sensation of weight or pull at the ear. The stimulus, which originated from an oscillator, was amplified by a constant-current amplifier. The intensity of the stimulus delivered to the subject was controlled by an attenuator. The wave was constantly monitored by an oscilloscope to see that it was sinusoidal.

Pain thresholds were determined by a modified method of limits, the 50-percent value being calculated. The method of limits was modified so that stimuli of an intensity very much in excess of threshold were not used. High-intensity stimuli of this nature are not only extremely unpleasant for the subject but they also produce such a persistent aftereffect that they mask subsequent stimuli. Even a stimulus of threshold intensity produced what was characteristically described as "stinging, nasty pain."

On the day after the last practice period, each subject was brought back for an additional session, when his pain threshold was determined. This usually required about 100 presentations of the electrical stimuli.

After the pain threshold had been determined, nine of the subjects were exposed, one at a time, to 4 days of sensory deprivation. A detailed description of the conditions of the confinement can be found in Vernon *et al.* (*2*). For the present, it is sufficient to say that sensory deprivation consisted of confinement in a small lightproof and soundproof cubicle. The cubicle was barely large enough to contain a single bed, upon which the subject lay. At the end of the 4 days of confinement and before he left the deprivation chamber, the subject's pain threshold was determined a second time.

The remaining nine subjects were not exposed to sensory deprivation, and thus they provided a control group. Four days after their pain thresholds had been determined they were called back to the laboratory, where a second determination of their pain thresholds was made. The pain thresholds for all subjects were determined in the sensory deprivation chamber under identical conditions. The major difference between the two groups was the manner in which they spent the 4 days which intervened between the first and second determinations of their pain thresholds. The experimental group spent this period under conditions of sensory deprivation, while the control group spent it in the normal manner.

The data for these two groups, presented in Table 1, reveal that, on the average, the pain threshold for the confined group dropped 0.108 ma after 96 hours of sensory deprivation. This change is statistically significant at better than the 1-percent level of confidence (*3*). All the experimental subjects showed a lowered pain threshold on the second determination. The control group showed a slight drop in pain threshold which averaged 0.019 ma—a change that is not significant. About half of the control group showed a rise in threshold, the other half, a drop. Thus, it can be seen that sensory deprivation not only has a uniform effect upon pain thresholds but also produces an increase in sensitivity. This is not the first instance of an increase in sensitivity resulting from isolation. Doane *et al.* (*4*) found the two-point limen to be significantly lowered after 48 and 72 hours of confinement.

One explanation of this finding is that a contrast phenomenon is produced by sensory deprivation. Another and more involved explanation can be formulated in terms of the action of the reticular formation of the brain stem. Under normal circumstances neural inputs from sensory departments can be blocked or partially inhibited at the level of the reticular formation. The blocking is produced in the descending tracts under cortical excitation aroused by any sensory stimulation. Thus it may be that sensory deprivation, by drastically reducing the amount of sensory input, minimizes the activity in the descending tracts of the reticular formation. If there is less inhibition to overcome at that level, then perhaps sensitivity is accordingly increased. In the case of pain, the neural impulses resulting from the pain stimuli would encounter less opposition and would register at a lower level of intensity. Or, to state it more accurately, registration would occur with impulses of lower frequency.

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Selective Viral and Rickettsial Serum Antibody Absorption by a Chromatographic Column

Abstract. Serum antibodies behave as cations at neutral *p*H and thus have low affinity for cellulose anion-exchange columns. Antigens of small size derived from adenovirus, influenza virus, and typhus rickettsiae, however, readily adsorbed to such columns. These adsorbed antigens specifically removed antibodies from antisera. This simple method permits antibody absorption by antigens ordinarily sedimented with difficulty.

Absorption of serum antibodies with particulate antigens has been a useful approach to the analysis of antigenic structure and antibody populations. This report presents a new method for antibody absorption which employs a chromatographic column to which "soluble antigens" are attached (adsorbed). Chromatographic columns, binding antigens covalently to resins or nonspecifically to other substances, have been employed to absorb and purify antibodies (*1*). Difficulties associated with this use of columns have been nonspecific antibody adherence to columns, antigen damage during chemical manipulations, and impair-

Table 1. Adenovirus type 7 serum (human, convalescent) before and after absorption by column antigens.

Column antigen	Serum CF titer with antigens*		
	Type 3	Type 4	Type 7
None	40	40	40
Adenovirus, type 7	<10	<10	<10
Adenovirus, type 4	<10	<10	<10

* Figures represent the reciprocal of the highest dilution of serum-fixing complement in the presence of four units of antigen.

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