Reports

Neutralization of Common Cold Agents in Volunteers by Pooled Human Globulin

The common cold has been transmitted repeatedly to volunteers by means of filtered nasal secretions (*1, 2*). Whether or not infected persons subsequently develop specific immunity to the common cold agents is not known. Some volunteers who developed a cold after receiving infectious nasal secretions have been observed to be susceptible to the same material at a later instillation. In addition, studies based upon spontaneous infections suggest that patients develop little or no immunity to the clinical common cold syndrome (*3*). Resistance to infection among adults, however, is greater than in children (*4*). Also, in a recent study the administration of vaccine made from a common cold agent isolated in tissue culture was believed to protect the subjects against this infection (*5*).

At the present time information about whether common cold agents elicit an antibody response in human beings and whether such an antibody can neutralize the infectious agents cannot be obtained from laboratory experiments. In the studies reported here (*6*), volunteers were challenged with infectious nasal secretions that frequently caused a common cold to develop, and pooled human gamma globulin was tested for its capacity to neutralize the infection.

Nasal secretions designated as NS142 and NS154, collected in different years from two persons with characteristic common colds, were filtered to remove bacteria and cells and stored at −70°C. In a search for known viruses, aliquots were tested on human epithelial cells, strain HeLa, and monkey kidney cells in tissue culture, in embryonated hens' eggs, and in suckling and adult mice. If no known viruses were found, the infectivity of the secretions was demonstrated by the transmission of an acute, afebrile, coryzal upper respiratory illness to volunteers by the instillation into each nostril of 0.2 ml of a 1:100 dilution of the nasal secretion in Hanks salt solution. Other subjects served as controls.

In each experiment four random matched groups of student nurses were given simultaneously one of the two secretions in one of a variety of diluents or an equal volume of diluent without nasal secretion. Uniform daily observations to detect the development of an experimental common cold were made for one week after the challenge, by an observer who was not informed regarding the nature of the instillation each volunteer had received. The details of the method and characteristics of the illness have been published elsewhere (*1*).

Neutralization of the infectivity of the nasal secretions was tested by incubating one part of a 1:10 dilution of a secretion with nine parts of the appropriate test diluent. The materials tested were pooled human globulin (*7*) in a final concentration of ± 8.0 g percent of protein, boiled human globulin concentrated to ± 12.0 g percent of protein, human serum albumin (*7*), or Hanks salt solution. The mixture was kept for 30 minutes at 37°C; then 0.2 ml was given to each volunteer as nose drops.

The results of seven replicate experiments in which the two different infectious nasal secretions were used with different lots of gamma globulin and human albumin are given in Table 1. The highest percentage of colds, 52 percent, occurred among volunteers challenged with nasal secretion diluted in buffered salt solution. Diluting the nasal secretion with the supernatant from a boiled gamma globulin solution did not alter the percentage of volunteers who developed an experimental cold. Similarly, mixing the nasal secretion in human albumin decreased the percentage of colds only slightly and insignificantly. When the infectious secretions were incubated with pooled human gamma globulin, however, colds were observed in only one-fifth as many subjects as in the two groups just described. Among the control subjects who received gamma globulin or diluent without infectious secretion, an average of 15 percent developed colds. "Spontaneous" colds among noninfected volunteers have been observed during previous experiments in approximately 10 percent of female subjects (*1*).

The distribution of experimental common colds among these groups is statistically highly significant ($p = < .001$). Among the groups inoculated with infectious nasal secretion in saline solution, boiled gamma globulin, or albumin, there was no significant difference. When the secretion was instilled in gamma globulin, the results were highly significantly different from the results with the preceding three vehicles ($p = < .001$) and were statistically similar to the results in the noninfected group. Pooled human gamma globulin was effective in delaying the appearance of symptoms among the volunteers who were not entirely protected. All of the individual symptoms were modified by gamma globulin.

With respect to the duration of the latent period before the onset of symptoms among the volunteers, the two infectious secretions, NS142 and NS154, behaved differently. Also, 90 percent of the symptoms produced by NS154 originated in the upper respiratory tract, whereas nearly 30 percent of those caused by NS142 were from the lower respiratory tree, or systemic symptoms. Paired serum specimens from the volunteers showed no antibody rise for influenza or adenoviruses or, with rare exception, significant neutralization of the new 2060 or J. H. agents (*8*).

The data show that the pooled human

Table 1. Transmission of colds to volunteers.

Challenge inoculum	No. of volunteers	No. with colds	Percent with colds
NS142	21	11	52.4
NS154	44	23	52.3
Subtotal	65	34	52.3
NS142 + boiled gamma globulin	20	11	55
NS154 + human albumin	18	8	44.5
Subtotal	38	19	50
NS142 + gamma globulin	22	3	13.7
NS154 + gamma globulin	37	3	8.1
Subtotal	59	6	10.2
Gamma globulin only	12	0	0
Hanks solution only	49	9	18.3
Subtotal	61	9	14.8

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globulin neutralized both agents and was approximately 65 percent effective in decreasing the mean symptom score caused by either one. Apparently these agents are antigenic in the human being, and they or related agents have caused rather widespread infections and antibody response. Nonspecific inhibition of the infectious agents is a possibility, but other experiments have discounted this (*3*).

One possible explanation of the lack of resistance against the common cold is that circulating antibody can protect against a systemic infection but is of little importance in the prevention of a local infection of the mucous membranes of the upper respiratory tract. We have shown that the common cold in adults is an afebrile and presumably localized respiratory infection (*1*), whereas children have more systemic symptoms. There is some evidence that local infections with other viruses also might occur in the presence of circulating antibody (*9*).

Prior studies in our laboratory have demonstrated that gamma globulin is consistently found in nasal secretions. It is not clear why the antibody is ineffective in the nose. The concentration of specific antibody might be critical, or inhibitory substances might block the antibody effect. Possibly cellular resistance is independent of antibody. Finally, antigenic variants of the agents that cause common colds might produce recurrent infection.

The demonstration of protective antibodies against the common cold in human gamma globulin is noteworthy with respect to the problem of immunity and immunization against this illness. Further exploration of some of the factors discussed is necessary before it will be known whether it is feasible to protect against common colds by the administration of vaccines.

GEORGE GEE JACKSON
HARRY F. DOWLING
TRUMAN O. ANDERSON
University of Illinois College of Medicine, Chicago, Illinois

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6. These studies were carried out under the sponsorship of the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiology Board and were supported in part by the Office of the Surgeon General, Department of the Army, and in part by grants from the Lederle Laboratories Division of the American Cyanamid Co. and from Charles Pfizer and Co., Inc. We gratefully acknowledge the assistance of Delores Seketa, Louise Jensen, and Marvin Turck, who participated in conducting the experiments.
7. The solution of pooled human plasma proteins was kindly furnished by Lederle Laboratories Division of the American Cyanamid Co., Pearl River, N. Y., and by Armour Laboratories, Chicago, Ill.
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6 March 1958

In vitro Decomposition of 1-Adrenochrome

1-Adrenochrome has been reported to produce psychotomimetic effects following intravenous injection (*1, 2*), and it has also been reported to be markedly elevated in the blood of normal subjects receiving lysergic acid diethylamide (*3*). In view of the reported instability of adrenochrome (*2, 4*) in the solid state and in solution prior to its injection, a study of the variables affecting decomposition was made (*5*).

1-Adrenochrome (*6*) was prepared as follows: To 176.5 mg of synthetic 1-epinephrine in 5 ml of redistilled methanol there was added 0.1 ml of 98-percent formic acid. One gram of freshly prepared silver oxide was added, with stirring. A deep red color developed immediately, and the temperature rose to 42°C. The reaction mixture was stirred for 2 minutes and centrifuged at high speed, and the supernatant solution was allowed to stand at −20°C for ½ hour. The precipitate was filtered and washed with a small volume of cold methanol-ether (1:1) followed by methanol-ether (1:3) and, finally, with ether to remove all traces of methanol. The yield of 1-adrenochrome was 54.3 mg (32.5 percent).

The absorption spectra showed three maxima, at λ-220, λ-300, and λ-480 mμ. The 480-mμ peak was used to follow the decomposition of 1-adrenochrome in 0.9-percent saline (*p*H 7.0), redistilled water (*p*H 7.0), and methanol solutions. It was found that the stability at room temperature (22°C) was greatest in sodium chloride solution and least in methanol solution. The decomposition was approximately 2.5 percent in saline, 5 percent in water, and 10 percent in methanol in 12 hours in the concentration range of 15 to 25 μg/ml and was approximately proportional to the time for at least 48 hours. It also was found that concentration affected the rate of decomposition. Thus, in saline solution, 3.8 mg of 1-adrenochrome per milliliter decomposed to the extent of 34 percent in 12 hours at room temperature, whereas, at 3.4 μg/ml, decomposition was not measurable even after 96 hours. Temperature also affected the decomposition. A solution of 1.9 mg of 1-adrenochrome per milliliter in 0.9-percent sodium chloride solution decomposed to the extent of more than 60 percent in 24 hours at room temperature but only to the extent of 4 percent at 3°C or at −20°C. The *p*H is also an important variable. Zambotti and Moret (*7*) reported that in 0.9-percent saline solution at 37°C in 2 hours there was a loss of 25 percent at *p*H 5.91 and of 90 percent at *p*H 7.38.

The decomposition of 1-adrenochrome in the solid state stored for 3 months at room temperature in an ordinary capped glass vial was also studied. No changes were observed in the infrared spectra obtained in the solid state in a potassium bromide pellet. The spectrum of the 1-adrenochrome synthesized by the method described was identical in all respects with the spectrum of a sample of 1-adrenochrome synthesized by the method of Sobotka and Austin (*8*), which was obtained from the Research Division of Armour and Co.

AARON FELDSTEIN
Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

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3 February 1958

Cytochemical Demonstration of Masked Lipids

There are several methods for the cytochemical or histochemical *in situ* demonstration of free lipids, or of lipids which give general or special color reactions (*1*). However, the larger fraction of lipids in ordinary cells (excluding fat cells or similar tissues) belongs to the class of, until now, cytochemically nondemonstrable or masked lipids, which should form lipoprotein complexes or complexes of a higher order. For instance, in calf-liver cells it has been possible, by extraction with suitable salt