rats fed a high fat diet did not result in an observable increase in the activity of this enzyme.

The loss of ornithine-transaminase activity was greater than the loss of liver protein in starved rats. This response to starvation is different from that of glutamate-oxalacetate transaminase which was conserved relative to liver protein, and also from that of glutamate-pyruvate transaminase, which increased relative to liver protein(2,3). This and other observations(1,6) indicate that the responses of different transaminases to a single treatment may be quite different.

Summary. The activity of ornithine-transaminase per g of liver from young rats fed an 80% casein diet for 4 days was from 4- to 8-fold the value for a control group fed a 25% casein diet. However, when mature or old rats were used in a similar experiment the response to increased protein intake was smaller or absent. Moderate restriction of food intake decreased the activity of the enzyme slightly, and starvation decreased it markedly. Feeding a low-carbohydrate, high-fat diet adequate in protein did not affect ornithine-transaminase activity.

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Antigenic Differences Between Two Strains of Respiratory Syncytial Virus. (28221)

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Respiratory syncytial virus (RS) is now recognized as one of the most important causes of severe respiratory tract illness in infants and young children(1-6). It was recently shown that hospital admissions for bronchiolitis or pneumonia in infants less than 6 months of age were 4 to 5 times greater during months of RS virus prevalence than during months when the agent was quiescent(6). There is, therefore, considerable interest in development of a vaccine against this agent. One important factor in preparing an effective vaccine is a thorough knowledge of the antigenic composition and extent of antigenic variation of the agent.

Early studies suggested that there was only one serotype of RS virus(1,7). However, we have reported that the A-1 strain of RS virus is not neutralized by hyperimmune guinea pig or rabbit serum prepared against the Long strain(8). Recently we have studied a strain of RS virus which differs even more from the Long strain. The nature and extent of this antigenic difference are reported here.

Materials and methods. Virus strains. The Long strain of RS virus was isolated in 1956 from a child with pneumonia(9). It was used in this study after 10 passages in human continuous cell lines (KB, Chang liver and HEp-2). The CH 18537 strain of RS virus was isolated during 1962 from a child with upper respiratory disease. This strain was

recovered in human lung diploid cell cultures (WI-26), then passaged 3 to 4 times in HEp-2 cell cultures(10). The CH 18537 strain was kindly provided by Drs. A. Vargosko and R. Parrott, Children's Hospital of D.C.

Virus assay was carried out in HEp-2 cell cultures as reported previously(8). Titers were calculated according to the method of Reed and Muench and expressed as 50% tissue culture doses (TCD_{50}) per ml(11). Ten-fold dilutions were prepared and 3 or 4 cultures inoculated per dilution.

Tissue cultures. HEp-2 cell cultures were grown in Eagle's medium containing 10% calf serum and maintained on Eagle's medium containing 5% chick serum. All sera were heat inactivated before use (56°C for 30 min). All media contained 2 times the usual concentration of amino acids and vitamins, 0.1 mM glutamine, 100 units of penicillin, 100 μg streptomycin, 5 μg amphotericin B and 50 μg kanomycin per ml. Cultures were incubated at 33-34°C in a stationary position.

Immune sera. Adult male ferrets were test bled and inoculated intranasally with 0.2 ml of virus while under sodium pentobarbital anesthesia. They were bled 3 to 4 weeks after inoculation, then challenged intranasally with the same virus. They were bled again 3 to 4 weeks after the second inoculation.

Acute and convalescent sera were available from young infants with serologic evidence of RS virus infection(5,12). These infants were hospitalized with a diagnosis of bronchiolitis or pneumonia during 1956-57, 1960 or 1962. Two criteria were used in selecting sera: 1) age of the child, and 2) development of neutralizing antibody during convalescence. It is probable that the development of antibody by these infants reflects the serologic response to primary infection with RS virus, since the infants from 1956-57 and 1960 were less than 8 months of age. In addition, the infants from the 1962 outbreak were born after the end of the 1961 epidemic (*i.e.*, during an inter-epidemic interval)(6). Sera were obtained from patients at the Children's Hospital of the District of Columbia with the exception of the 1956-57 sera which were from infants admitted to Baltimore City Hospital(12). All sera were stored at -20°C.

Serology. Neutralization tests were carried out as described previously(4). Four-fold dilutions of heat inactivated serum (56°C for 30 min) were incubated at room temperature for one hour with an equal volume of diluted virus containing approximately 100 TCD_{50} per 0.1 ml. Two-tenths ml of serum-virus mixture was inoculated into each of 2 to 4 HEp-2 cell cultures. Tests were read after 3 to 4 days incubation when simultaneous titrations showed the presence of 32 to 100 TCD_{50} of virus. Reading of the tests was facilitated by adding neutral red at a final dilution of 1:80,000 to the tissue culture medium(13).

The technique for the complement-fixation (CF) test and the preparation of Long CF antigen have been described(14). The CH 18537 antigen was prepared in HEp-2 cell cultures and consisted of infected cells and tissue culture fluid harvested when the entire cell sheet showed cytopathic effect. This material was frozen and thawed once and then stored at -60°C until used.

Soluble CF antigen consisted of the top 10 ml of supernatant fluid after centrifugation of 30 ml of infected tissue culture fluid at 30,000 rpm for one hour in a Spinco #30 rotor. Data on the separation of CF activity and infectivity are presented under *"Results."*

Growth of virus in ferrets. Ferrets were inoculated intranasally as described previously. Animals were killed daily thereafter. The turbinates were removed and a 10% weight to volume homogenate prepared in Hanks' balanced salt solution with 0.5% gelatin using a Ten Broeck tissue grinder. The quantity of virus in the homogenate was titrated immediately.

Results. Comparison of strains in neutralization tests—a) using ferret immune sera. Reciprocal neutralization tests were carried out using serum from ferrets infected with the Long and CH 18537 strains of RS virus. Following primary infection all of the 4 animals inoculated with the Long strain developed an homologous level of neutralizing antibody which was 4-fold or more higher than that against the CH 18537 strain (Table I). In addition the 4 ferrets inoculated with the CH 18537 strain developed antibody to

TABLE I. Antigenic Differences Between 2 Strains of Respiratory Syncytial Virus.

		Reciprocal serum neutralizing antibody titer with indicated virus					
		Long (1956 isolate)			CH 18537 (1962 isolate)		
Post infection ferret serum	Animal No.	Pre-infection	Post-infection*	Post-re-infection†	Pre-infection	Post-infection*	Post-re-infection†
Long‡	1	<8	512	512	<8	32	256
	2	"	32	64	"	<16	32
	3	"	128	64	"	<16	32
	4	"	128	32	"	16	32
CH 18537§	5	<4	6	12	<4	18	192
	6	"	<4	12	"	64	64
	7	"	"	8	"	48	22
	8	"	"	<4	"	32	48

* 3 (CH 18537) or 4 (Long) weeks following intranasal infection.
† 3 (Long) or 4 (CH 18537) weeks following a second intranasal inoculation.
‡ 2 HEp-2 cultures used for each 4-fold dilution.
§ 4 HEp-2 cultures used for each 4-fold dilution.

the infecting virus, whereas 3 of these animals failed to develop antibody to the Long strain. Furthermore, high titered rabbit hyperimmune serum for the Long strain had little or no neutralizing activity for the CH 18537 strain (Table II). These findings indicate that reciprocal antigenic differences exist between the 2 strains. The results cannot be explained by strain differences in avidity for antibody.

Three to four weeks after primary inoculation the ferrets were challenged by intranasal inoculation with the homologous strain of the virus. The animals inoculated with the Long strain showed no change in homologous titer; however, in 3 instances the heterologous titer increased to approximate the homologus titer. One of the 4 animals reinoculated with the CH 18537 strain failed to develop heterologous antibody, while 2 other ferrets developed low level heterologous responses. One of the 4 animals had a rise in homologous antibody titer following reinoculation with the CH 18537 strain.

TABLE II. Comparison of 2 Strains of Respiratory Syncytial Virus Using Paired Sera of Children with Bronchiolitis or Pneumonia.

			Reciprocal serum neutralizing antibody titer against indicated antigen*			
Infected individual			Long (1956 isolate)		CH 18537 (1962 isolate)	
Year	Age in mo	Test No.	Acute	Conv.	Acute	Conv.
1956	1†	2	4 or <	32	4 or <	128
	3	2	16	24	8	192
	4	1	3 or <	12	3 or <	12
	4	1	3 or <	192	3 or <	48
	6	1	3 or <	48	12	768 or >
	7	1	3 or <	48	3 or <	96
	7	2	4 or <	48	4 or <	48
1960	5	1	3 or <	48	3 or <	48
	7	1	3 or <	48	3 or <	12
1962	9	2	4 or <	16	8	16
	10	1	3 or <	48	3 or <	24
	11	1	3 or <	24	3 or <	48
	11	2	4 or <	16	16	256
	12	2	4 or <	16	4 or <	32
	14	2	4 or <	16	4 or <	64
Control rabbit		1		384		3 or <
Hyperimmunized against Long strain		2		512		8

* Serum neutralizing antibody titer determined against 100-320 TCD_{50} of indicated virus.
† Control with no history of illness.

TABLE III. Separation of Infectivity and Complement-Fixing Activity of 2 Strains of Respiratory Syncytial Virus by Centrifugation (30,000 rpm, 1 Hour).

Antigen		Virus titer in $TCD_{50} \times 10^3$/ml	CF activity in units/ml	TCD_{50}/CF unit $\times 10^2$
Long	Pellet*	830	13	640
	Supernatant†	32	320	1
CH 18537	Pellet	132	6	220
	Supernatant	.8	320	.025

* Calculated for resuspended pellet.

† Top 10 ml of supernatant fluid taken from a #30 rotor cup filled with 30 ml of virus suspension.

These results indicate that ferrets, when reinoculated with the homologous strain of RS virus 3 to 4 weeks after primary infection, may become reinfected even though they have relatively high levels of neutralizing antibody to the inoculated virus. In the majority of instances this reinfection is manifest by a broadening of the antibody spectrum with little or no change in the homologous antibody titer.

b) Using paired human sera. Neutralization tests were carried out using acute and convalescent sera from children with bronchiolitis or pneumonia. Only one infant 3 months of age had neutralizing antibody in the acute phase serum when tested with both strains of RS virus; in addition, 3 infants, 6, 9 and 11 months old, respectively, had low levels of neutralizing antibody for the CH 18537 strain. With 2 exceptions the children developed significant levels of neutralizing antibody to both strains of RS virus (Table II). The 3-month-old mentioned above failed to develop a rise in neutralizing antibody for the Long strain, although the antibody titer for the CH 18537 strain rose to a level of 1:192. The second exception involved a 9-month-old with pre-existing neutralizing antibody to the CH 18537 strain. This infant failed to develop a rise in antibody to that strain, whereas he developed a low level response (1:16) for the Long strain. In the majority of individuals the convalescent phase antibody titers were similar when measured with the two strains. However, 3 of the 4 children with pre-existing neutralizing antibody for the CH 18537 strain developed a higher level of neutralizing antibody for that agent than for the Long strain.

Comparison of strains in complement fixation tests. It was demonstrated previously that the complement-fixing antigen of RS virus is soluble and can be separated from the viral particle by centrifugation at 30,000 rpm for one hour(9). The existence of an antigen separable from the virus particle made it possible to compare the 2 RS virus strains by a technique other than neutralization. Soluble antigens were prepared for the CH 18537 and Long strains of RS virus as described in "*Methods.*" Almost all of the infectivity was found in the pellet following centrifugation, while the supernatant fluid contained a large quantity of CF antigen relatively free of infective virus (Table III).

When the soluble CF antigens of the Long and CH 18537 strains were tested against paired human sera (Table IV) significant CF antibody responses were detected with the antigens of both strains. Dilutions of serum above 1:64 were not tested since previous tests indicated that these convalescent sera did not fix complement at a dilution of 1:128.

Since supernatant fluid preparations contained insufficient antigen to give more than trace CF reactions with immune ferret serum comparative tests were performed using a suspension of infected tissue culture cells and fluid as antigen. Table V shows the results of checkerboard titrations carried out with post infection ferret sera. There were no differences between CF antigens prepared from the 2 strains. Tests performed with other sera obtained at different times after infection of ferrets yielded similar results.

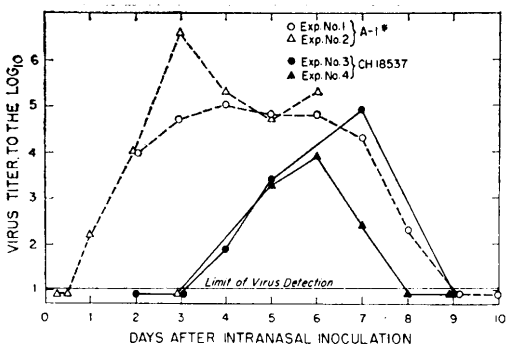
Growth curve of CH 18537 in the ferret turbinate. Ferrets were inoculated intranasally with approximately $10^{2.5}$ TCD_{50} of the CH 18537 strain. At intervals from the 2nd to the 12th day after inoculation single ani-

TABLE IV. Relationship Between Soluble Complement-Fixing Antigens of 2 Strains of Respiratory Syncytial Virus Using Paired Human Sera.

Infected individual		Reciprocal serum antibody titer against the indicated antigen					
		Long supernatant*		CH 18537 supernatant*		Long CF antigen†	
Year	Age (mo)	Acute	Conv.	Acute	Conv.	Acute	Conv.
1958	27	<8	64 or >	<8	64 or >	<8	64 or >
1960‡		<8	32	<8	32	16	64 or >
1961	19	<8	64 or >	<8	64 or >	<8	64 or >
1962	18	<8	64 or >	<8	64 or >	8	64 or >

* 30,000 rpm (1 hr) supernatant fluid.
† Untreated antigen consisting of infected tissue culture cells and fluid.
‡ Adult volunteer.

mals were killed and the amount of virus present in the nasal turbinate titrated in HEp-2 cell cultures as described. Virus was not recovered before the 4th day after inoculation (Fig. 1). Maximum levels of virus were present on the 6th and 7th days after infection. Shortly thereafter the quantity of virus rapidly decreased and it could not be detected on the 8th to 9th days.



* Data from a previous study. Coates and Chanock(8).

FIG. 1. Virus recovery in TCD_{50}/0.1 g of turbinate of ferrets infected intranasally with respiratory syncytial virus.

In Fig. 1 the growth of the CH 18537 strain in the ferret turbinate is compared with that of the A-1 strain. The latter data are taken from a previous study(8). It appears that the CH 18537 strain has a longer latent period in the ferret than does the A-1 strain. The A-1 strain was recovered from the 1st to the 8th day after inoculation, with the peak of virus recovery on the 3rd to 4th day after infection. The animals in the previous study were inoculated with from $10^{2.3}$ to $10^{2.7}$ TCD_{50} of the A-1 strain(8).

Discussion. Reciprocal antigenic differences between 2 RS virus strains were demonstrated in tests with post infection ferret serum. Ferrets infected with the Long or CH 18537 strains generally developed higher levels of neutralizing antibody for the infecting strain. Differences between the 2 strains were minimal, however, when the viruses were

TABLE V. Relationship of Complement-Fixing Antigens of Two Strains of Respiratory Syncytial Virus.*

Post infection ferret serum‡	Serum dilution	Dilution of indicated antigen†							
		Long				CH 18537			
		1:1	1:2	1:4	1:8	1:1	1:2	1:4	1:8
Long	1:10	4	4	3	2	4	4	3	2
	1:20	2	2	1	trace	3	2	1	trace
	1:40	trace	trace	trace	0	trace	trace	trace	0
	1:80	0				0			
CH 18537	1:10	4	4	3	1	4	4	3	1
	1:20	4	4	3	1	4	4	3	trace
	1:40	4	4	2	0	4	4	3	"
	1:80	3	2	1		4	4	2	0
	1:160	1	0	0		2	1	trace	0

* 4 = complete fixation of complement; 0 = no fixation.
† Untreated antigen consisting of infected tissue culture cells and fluid.
‡ 3 (CH 18537) or 4 (Long) weeks following intranasal inoculation.

used to assay the antibody response of infants infected with RS virus during 1956, 1960, and 1962. Three explanations for the disparity in serologic response of children and ferrets, both presumably undergoing primary infection with RS virus, can be suggested: 1) the antibody response of ferrets may be more specific than that of infants, 2) RS virus, a human agent, may replicate more extensively and to a higher level in infants than in ferrets, thus providing an opportunity for a broader antibody response, and 3) it is possible that the infants in this study were infected with a related as yet unrecognized strain of RS virus which shares antigenic components with both the Long and the CH 18537 strains.

Of these the third possibility seems least probable since one must also postulate that the hypothetical related strain of RS virus shares an approximately equal number of antigenic determinants with both the Long and the CH 18537 strains. The 2nd possibility is favored by the observation that lower respiratory tract involvement occurs commonly in infants, whereas it has not been observed during infection of ferrets(4,8). Thus in infants it is possible that a greater quantity of lymphoid tissue is stimulated to antibody production by the virus than is the case in the ferret where infection is localized in the upper respiratory tract.

The CH 18537 strain grew more slowly in the ferret turbinate than did the A-1 strain, although the quantity of virus inoculated was the same. These findings suggest that not only is the CH 18537 strain of RS virus antigenically different from the Long strain, but also that it is biologically different from the A-1 strain. The A-1 strain was previously shown to be antigenically different from the Long strain when neutralization tests were carried out using hyperimmune rabbit or guinea pig serum, but not when post infection ferret serum was used(8).

The present findings represent the first demonstration of antigenic variation of RS virus. At this early stage in our understanding of such antigenic variation many questions can be raised. First, what is the extent and breadth of antigenic difference among RS viruses? How many such variants exist and what is the time sequence of variation? Do variants simultaneously circulate in human populations or is variation discontinuous as observed during major antigenic shifts among influenza A viruses(15)? One observation from this study suggests that variants may circulate simultaneously in the same population. Thus, 2 infants infected during 1956, the year the Long strain was recovered, developed antibodies which were more reactive with the CH 18537 strain than with the former strain.

Rabbits inoculated parenterally with RS virus do not appear to become infected. When these animals are hyperimmunized by parenteral inoculation of the Long strain, they develop little or no antibody for the A-1 or CH 18537 strains of RS virus(8). Will a similar sequence of events be observed following inoculation of infants and children with inactivated RS virus vaccine? Will it be necessary to include several different strains in order to prepare an effective RS vaccine? It is clear that answers to these questions must be available before a meaningful approach to immunoprophylaxis can be made.

Summary. A strain of RS virus (the CH 18537 strain isolated during 1962) was demonstrated to differ antigenically from the Long strain (isolated during 1956). This difference was reciprocal when neutralization tests were carried out using post infection ferret serum. Reinoculation of the ferrets with the Long and CH 18537 strains generally resulted in reinfection which was manifest by a broadening of the antibody spectrum with little or no change in the homologous antibody titer. In addition the growth of the CH 18537 strain in the ferret turbinate was studied and found to be different from that of the A-1 strain of RS virus. Differences between the Long and the CH 18537 strains of RS virus were minimal when the viruses were used to assay antibody response of infants infected with RS virus during 1956, 1960 and 1962. However, 2 infants infected with RS virus during 1956 developed antibodies which were more reactive with the 1962 isolate. It was suggested that variants of RS virus may circulate simultaneously in the same human population. The pertinence of these findings to

development of immunoprophylaxis was discussed.

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Effect on Tumor Homografts of Treating Hosts with Antiproteolytic Enzyme Compounds.* (28222)

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The exact role of proteolytic enzymes in delayed hypersensitivity reactions is not clearly defined and has stimulated controversy. Recently, however, studies utilizing antiproteolytic enzyme factors established that proteolytic enzymes are at least partially responsible for the development of some hypersensitivity responses. Substances such as epsilon amino-n-caproic acid (EACA)(1) and soy bean trypsin inhibitor (SBTI)(2) suppress proteolytc enzymes of the chymotrypsin type. Zweifach(3) demonstrated that EACA modifies both the reaction to solubilized antigen-antibody complex and the cutaneous reaction to mixtures of endotoxin and epinephrine. It also exerts a protective effect against acute endotoxemia and Noble Collop drum shock. Chryssanthou(4) showed that SBTI can abrogate the delayed Schwartzmann reaction. Recent experiments in our laboratory using these compounds indicated that enzymes of the chymotrypsin type also participate in the rejection of homografts of normal tissue(5). Specifically, administration of EACA to host mice attenuated the rejection reaction as evidenced by the extended survival time of skin homografts and the modified cytologic reactions observed in the host bed.

Tumor immunity differs in several respects from normal tissue immunity. For example, the enhancement phenomenon can be produced with certain types of tumor tissue indicating that different mechanisms are involved. Therefore, we decided that treating animals that have received tumor homografts with EACA and SBTI might further delineate the role of proteolytic enzymes in the mechanisms of rejection.

Materials and methods. A mammary adenocarcinoma, *H2712*,† which is strain spe-

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