tase is confined to the islet tissue and that its mode of action differs somewhat from that of mammalian elastase. The latter may be due to species differences in the enzyme or the substrate, or both. The fact that elastase may be found in such divergent forms as fish and mammals indicates need for further study of the phylogenetic distribution of this enzyme and of a possible basic function for elastase. Experiments are currently being conducted to determine if elastase is also a component of the islet tissue of mammals.

Summary. Islet tissue of the teleost fish, *Lophius piscatorius,* contains elastase whereas acinar tissue seems to be free of this enzyme.

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Received November 23, 1953. P.S.E.B.M., 1953, v84.

Antigenic Differences Among Strains of Newcastle Disease Virus.* (20754)

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Newcastle disease, first recognized in the Dutch East Indies in 1926, is now known throughout the poultry raising regions of the world. Described in the Far East as a highly fatal infection of chickens, the virus evaded early recognition in many regions by appearing as a mild respiratory disease. In spite of the variations in pathogenicity and in other properties, most investigators have found that strains of the virus whether isolated in Asia, Africa or America are antigenically homogeneous(1,2). Contrary evidence is scanty and based on the relative immunogenicity of vaccines(3,4). The repository of strains of Newcastle disease virus assembled at the University of Wisconsin under the auspices of the Bureau of Animal Industry and the North Central Regional Technical Committee on Newcastle Disease provided material for an investigation of antigenic diversity among 13 strains of the virus. The cultures available had been isolated in many localities in the United States, Canada, and Mexico and from a few places in Europe and Australia over a period of 20 years.

* Published with the approval of the Director of the Wisc. Exp. Station Paper NS 127.

Methods. The relative abilities of homologous and heterologous antisera to neutralize the embryo infectivity of the virus were compared. The immune sera were obtained from chickens and rabbits about 21 days after injection of virus and used unpooled. The rabbit serum was inactivated at 56°C for 30 minutes and chicken serum was used uninactivated. Infected allantoic fluids kept at −20°C were used as the virus source. Serial ten-fold dilutions of virus which had been incubated 30 minutes at room temperature with undiluted antiserum were inoculated intrallantoically in 0.1 ml amounts into 10- or 11-day embryonating eggs. Six eggs were used for each dilution, and all eggs candled twice daily for 7 days. The number of 50% lethal doses of virus neutralized by each serum was calculated by the procedure described by Smadel(5). On the basis of replicate tests prepared simultaneously by 2 individuals using the same stock virus and same serum, a difference of greater than one log was considered significant, 0.6 to 1.0 log of possible significance and 0.5 log or less of no significance (Table I). To keep the amount of non-specific difference at a minimum, heterologous serum-virus combinations were always compared to a homolo-

TABLE I. Comparison of Serum Neutralization (SN) Titers Obtained by 2 Individuals Employing Same Stock Virus and Serum and Performing the Test on the Same Day.

Virus	Serum	Log SN titer Test A	Test B
125-Iowa	125-Iowa	4.3	4.4
	11914 FD	3.3	3.5
	Naj	7.4	7.5
	RO	3.4	3.5
11914 FD	11914 FD	4.7	5.2
	Naj	5.0	5.0
	RO	4.7	4.7
	Vict	4.7	5.0

TABLE II. Cross Serum Neutralization (SN) Reactions with 5 Strains of Newcastle Disease Virus.

Virus	Serum RO	Naj	Mil	125-Iowa	11914
RO		6.0–5.9*	6.6–4.8		6.4–6.7
		7.9–6.7			6.0–6.7
Naj	6.7–3.0		6.6–5.3	6.4–6.3	
			6.0–5.8†	6.4–6.8†	
Mil	6.7–1.8	7.9–4.0			
	6.7–1.9	7.7–5.3			
	7.8–1.7	7.8–5.2†			
		7.8–5.1†			
125 Iowa		6.2–6.0			
		6.5–5.5			
		7.9–7.4			
		7.8–7.1†			
11914	6.7–4.7				
Homologous relative titer‡ (homologous titer — heterologous titer) = heterologous relative titer.					
RO	8.0	7.4	6.4		8.5
Naj	4.3	8.0	7.3	8.1	
Mil	2.8	5.1	8.0		
125-Iowa		7.7		8.0	
11914	6.0				8.0
		% relationships§			
RO	100				
Naj	68	100			
Mil	52	76	100		
125-Iowa		100		100	
11914	88				100

* Logarithm of the LD_{50} of virus neutralized by the homologous serum-logarithm of the LD_{50} of virus neutralized by the heterologous serum.

† Chicken sera, all other sera obtained from rabbits.

‡ The homologous titers rounded to 8; the highest whole number.

§ The cross reaction was determined by the application of the formula $R = \sqrt{r_1 \times r_2}$ to the logarithm of the titers and then expressed in %.

gous serum-virus combination prepared and tested at the same time.

Results. Significant differences were detected in 3 of 5 antiserum-virus reciprocal combinations (Table II). The differences (determined by the formula $R = \sqrt{r_1 \times r_2}$ of Archetti and Horsfall(6)) were expressed as 76, 68 and 52% relationship. A pair of viruses neutralizing in one direction and not in the other would be expressed as 50% relationship.

The RO antiserum, which detected greatest differences among a few strains was subsequently used in neutralization tests with 12 strains (Table III). The greatest serological diversity observed existed between the RO strain from California and 3 strains from the eastern hemisphere, Herts and Milano of Europe and Vict of Australia. One American strain, 125-Iowa, however, exhibited as much divergence from strain RO as did Herts. Serological differences may be of considerable importance as exemplified by the results obtained with RO antisera and Milano virus, *e.g.*, homologous combination, 7.8 embryo LD_{50} neutralized; heterologous combination, 1.7 embryo LD_{50} neutralized. In such instances the relationship of the pair would not be accepted by diagnostic laboratories as significant.

Differences between strains were frequently greater when one of the pair was used as the antigen than when the other was employed

TABLE III. Per Cent Relationship of 12 Strains of NDV as Determined by Use of RO Antiserum* in the Serum Neutralization Test.

Virus	Titer†	Difference	Relationship‡
RO	7.4–7.1	.3	96
Bl	7.1–6.6	.5	93
Roakin	6.7–5.1	1.6	76
Ixta	7.1–5.2	1.9	73
GB	7.1–5.2	1.9	73
11914	6.7–4.7	2.0	70
MD I	7.4–5.1	2.3	69
KM	7.4–4.4	3.0	60
Naj	6.7–3.7	3.0	55
125-Iowa	6.7–3.4	3.3	50
Herts	7.0–3.7	3.3	52
Vict	6.7–2.9	3.8	43
Milano	6.7–1.8	4.9	27
Virus and normal serum	6.7–1.5	5.2	22

* Prepared in rabbits and inactivated.

† Logarithm of the LD_{50} neutralized by homologous serum — logarithm of the LD_{50} neutralized by heterologous serum.

‡ $\frac{\text{Heterologous titer}}{\text{Homologous titer}} \times 100 = \text{relationship.}$

(Table II). A few strains appeared to be similar in one cross: 11914 antisera with RO virus (6.4; 6.7) and quite different when the reciprocal cross was used, RO antisera with 11914 virus (6.7; 4.7). Sometimes the neutralization titer appeared to be greater using a heterologous serum than a homologous one (11914 antisera and RO virus) (6.4; 6.7). Strain 11914 with which this occurred most frequently has been found to protect chickens better against challenge with certain other strains than against itself(3). The sensitiveness of individual sera in detecting antigenic differences varied considerably, (Table II), but in general, chicken and rabbit antisera were equally sensitive in detecting differences among strains of NDV. The study of the antigenic diversity of strains of Newcastle disease viruses by the serum neutralization test while still in preliminary stages has revealed differences of diagnostic importance, and raises a question of the feasibility of using a single vaccine in all situations.

Summary. Seventeen heterologous combinations of Newcastle disease virus and antiserum have been used in serum neutralization tests. Of these combinations 13 exhibited a difference of greater than 1.0 log between the neutralization of the homologous and the heterologous virus by prepared antiserum. A difference of 5.0 logs was observed between 2 strains.

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Received November 12, 1953. P.S.E.B.M., 1953, v84.

Hemolysis of PR8-Virus Sensitized Erythrocytes.* (20755)

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Microbial components may be roughly separated into 2 general groups with respect to their ability to agglutinate erythrocytes of various species. One, consisting of various viruses and bacterial products, exhibits the well-known Hirst phenomenon or direct hemagglutination(1,2); and the other, consisting usually of polysaccharide components of various bacteria, sensitizes erythrocytes of various species to agglutination by antisera homologous to the adsorbed microbial components(3-5). In the case of the former group(6) and of the latter group(5,7-10) it has been found by several workers that following the addition of antiserum homologous with the adsorbed microbial component, the addition of complement (C′) brings about lysis. This type of hemolytic system, therefore, differs from the usual complement-fixation system in that the antibody is directed not toward the erythrocyte but to the adsorbed component.

One phase of a related problem in which we are interested directed our attention to a study of the conditions under which the influenza virus could be involved in the lysis of erythrocytes. A survey of the literature available to us failed to reveal any reports upon the lysis of influenza virus-sensitized erythrocytes under the influence of C′, in spite of the generally accepted procedure of inactivating sera before use in the hemagglutination-inhibition test(11). This report will describe some results obtained from such a study.

Methods. Veronal buffer, pH 7.4(12) was

* This investigation was supported in part by a research grant from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.