cretion of pancreatic juice of high enzyme concentration, and that insulin hypoglycemia may excite vagus centers. The latter has been proved abundantly by studies on gastric secretion and motility. It may be assumed that the excitatory effect of insulin on the secretion of pancreatic enzymes is due to a generalized hypoglycemic excitation of visceral centers, including those of the vagus.

Summary. Healthy human subjects were given 2 or 3 hourly intravenous injections of secretin. In some of the experiments insulin was given intravenously during the course of the pancreatic secretion evoked by the secretin. It was found that during insulin hypoglycemia the concentration and output of pancreatic enzymes was increased but that the volume of secretion was not influenced.

16899

Heat Stability of Hemagglutinin of Various Strains of Newcastle Disease Virus.*

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Determination of the heat stability of the hemagglutinin was selected as the first method of approach to the problem of characterizing the antigenically homologous strains[1] of Newcastle (NDV) virus. A method of identifying strains was deemed necessary for proposed studies of virulence and immunogenicity of the virus. For purpose of survey, 24 strains of virus obtained from widely distributed points in North America and Europe were cultured in embryonating eggs and the stability of the hemagglutinin (HA) to heating at 56°C determined.

The heat stability of the influenza hemagglutinin[2] has been extensively studied. It was the demonstration by Salk[3] of differences in heat stability of the influenza strains which influenced our choice of approach. Scott and Lauffer[4] described the inactivation of hemagglutinin of influenza virus as a first order reaction by assuming a multicomponent system. The demonstration that the site of inactivation of the hemagglutinin and the site of infectivity for embryos were independent was, therefore, not surprising.[5] The Henles[6] have shown that with heat and ultraviolet treatment, certain properties of influenza virus can be arranged in a descending order of susceptibility to inactivation. The capacity to infect embryos was lost first, then, in order, the ability to interfere with infection, to elute from agglutinated red blood cells, to agglutinate red blood cells, and subsequently, the ability to fix complement in the presence of immune sera. Antigenicity could not always be fitted into this order, but sometimes it persisted until after the hemagglutinin had been destroyed. With certain strains of influenza virus Salk[7] demonstrated the protec-

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1 Brandly, C. A., Moses, H. E., Jungherr, E. L., and Jones, E. E., *Am. J. Vet. Res.*, 1946, **7**, 289.

2 Burnet, F. M., *Aust. J. Exp. Biol. and Med. Sci.*, 1942, **20**, 81.

3 Salk, J. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 134.

4 Scott, E. M., and Lauffer, M. A., *Arch. Biochem.*, 1946, **11**, 185.

5 Lauffer, M. A., Carnelly, H. L., and MacDonald, E., *Arch. Biochem.*, 1948, **16**, 321.

6 Henle, W., and Henle, G., *J. Exp. Med.*, 1947, **85**, 347.

7 Salk, J. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 140.

TABLE I.
Description of Strains.

Place of isolation	Date of isolation	Donor	Source animal	Code
New Jersey	1944	F. R. Beaudette	Chicken	N. J. (NJ-KD)
?	?	F. R. Beaudette	?	Beaudette vaccine (FRB)
New York	45	P. P. Levine	Chicken	N. Y. Levine (NYL)
New York	46	J. Fabricant	Turkey	Mayer turkey (NYT)
Massachusetts	45	C. A. Brandly	Chicken	Huntington Lab (4F)
Iowa	46	M. A. Hofstadt	Chicken	23 E 219 (HOF)
Michigan	46	C. H. Cunningham	"	46-967 (MIC)
Missouri	47	H. C. McDougle	"	31747 (31747)
Kansas	48	L. D. Bushnell	"	Leavenworth (KL)
Kansas	48	L. D. Bushnell	"	Manhattan (KM)
Wisconsin	46	Field	"	Lancaster 1660 (WL)
Wisconsin	48	Field	Turkey	Henry 24156 (HHT56)
Minnesota	47	B. S. Pomeroy	"	(TM)
California	44	J. R. Beach	Chicken	(11914)
Canada	47	R. V. L. Walker	"	Allen M4947 (ALL)
"	47	R. V. L. Walker	"	Berwick 29507 (BER)
"	47	R. V. L. Walker	"	Cleroux M4969 (CLE)
"	47	R. V. L. Walker	"	Herr M5004 (HER)
"	47	R. V. L. Walker	"	Labelle M4970 (LAB)
"	47	R. V. L. Walker	"	McVey M5016 (McV)
"	47	R. V. L. Walker	"	Watford No. 5 (WAT)
"	47	R. V. L. Walker	Turkey	Peerless M4945 (TP)
England	33	Min. of Agric.	Chicken	Hertfordshire (E)
Italy	45	U. S. Army	"	Milano (M)

tive action of traces of formalin to the denaturation process. Francis[8] reported the divergence of the capacity to show inhibition in the presence of immune sera when the hemagglutinin was heated.

Methods. For ease of comparison and for the sake of simplicity the methods used follow those for similar work with influenza virus insofar as the properties of Newcastle virus permit.

Virus material from an early passage of each strain (less than 10 egg passages) was utilized to avoid changes which may develop in the character of the strain on prolonged cultivation of Newcastle virus in the embryonating egg.[1,9-12] Only freshly harvested virus or material held for less than one month at —20°C was used for determinations. Allanto-amnionic fluids become less stable to heat treatment after storage at 4°C, but no significant changes were observed after storage at —20°C for the periods and strains studied.

Clear allanto-amnionic fluids from moribund or dead embryos were sealed in 5 ml lyophilizing vials by drawing out the necks of the vials in a flame. The vials were submerged in a constant temperature water bath at 56 ± 0.5°C, the water being agitated constantly with an electric stirrer. Vials were removed at selected intervals and immediately chilled in ice water. Pooled allanto-amnionic fluids from four to eight eggs were used for all determinations. Heat stability data were obtained on at least two pools and in most instances four or five pools of each strain. Virus pools of a given strain, but not necessarily individual egg fluids of a given strain, gave closely reproducible results. Reasons for individual egg variations have been explored, and with certain precautions observed in har-

[8] Francis, T., Jr., *J. Exp. Med.*, 1947, **85**, 1.

[9] Iyer, S. G., and Dobson, N., *Vet. Rec.*, 1941, **53**, 381.

[10] Iyer, S. G., and Hashni, Z. A., *Indian J. Vet. Sci.*, 1945, **15**, 155.

[11] Winslow, N. S., 1947. Thesis submitted for degree of Master of Science, University of Wisconsin.

[12] Beaudette, F. R., Proc. 47th Ann. Meet. U. S. Livestock San. Assoc., 1943, 122.

TABLE II.
Heat Stability of the Hemagglutinin at 56°C.

Strain	Minutes*						
	0	15	30	60	120	240	480
NJ-KD	1280	0	0	0	0	0	0
FRB	1280	0	0	0	0	0	0
NYL	1280	20	0	0	0	0	0
NYT	1280	10	0	0	0	0	0
4F	1280	160	10	0	0	0	0
HOF	1280	160	20	0	0	0	0
MIC	1280	640	40	0	0	0	0
31747	1280	320	40	0	0	0	0
KL	1280	1280	1280	1280	320	160	0
KM	1280	1280	1280	640	320	5	0
WL	1280	40	0	0	0	0	0
HHT56	1280	0	0	0	0	0	0
TM	1280	1280	640	640	160	10	0
11914	1280	320	160	10	0	0	0
ALL	1280	1280	1280	640	320	160	0
BER	1280	1280	1280	640	320	20	0
CLE	1280	1280	1280	640	80	20	0
HER	1280	1280	1280	1280	640	160	0
LAB	1280	1280	640	640	320	10	0
McV	1280	640	320	160	20	0	0
WAT	1280	1280	640	40	0	0	0
TP	1280	1280	1280	640	320	40	0
E	1280	160	20	0	0	0	0
M	1280	1280	1280	640	80	20	0

* Data obtained at different time intervals (see text) are not included in table.

vesting fluids, close correlation can be obtained among individual egg fluids containing virus of a given strain.

The virus pools ranged between pH 7.9 and pH 8.2. There was no relationship between pH of the fluids on harvest and stability of the virus in the fluid to heating.

The hemagglutination test procedure substantially followed that outlined by Salk.[13] A 0.2 ml volume of virus was added to 0.8 ml of saline in agglutination tubes (14.5 × 75 mm). Five-tenths of a milliliter of this 1:5 dilution was transferred to 0.5 ml of saline, thoroughly mixed, and similar transfers repeated until a 1-1280 dilution was obtained. Twenty-five hundredths milliliters of 1% fowl red blood cells was added to all tubes and the rack shaken well. The hydrogen ion concentration of the virus-saline-red cell mixtures ranged from pH 7 to 8. The test was incubated at room temperature of 25 to 30°C and read from 15 to 45 minutes later,[14] the period of reading being determined by the controls. The endpoint was considered to be a definite one plus agglutination. The effect on titers of differences in the agglutinability of red cells from one fowl to another was considerably resolved by using the highly agglutinable cells of a single fowl as a standard for all tests.[14]

Experimental. A description of the 24 strains is given in Table I. The localities of isolation are roughly grouped into 5 regions, eastern United States, the middle western United States, western United States, Canada, and Europe. The dates of isolation range from 1933 to 1948, a period of 15 years. Four of the strains were obtained from turkeys and 20 from chickens. The abbreviated code given in parentheses following the complete strain designation is used in the text and accompanying figures.

[13] Salk, J. E., *J. Immunol.*, 1944, **49**, 87.

[14] Brandly, C. A., Hanson, R. P., Lewis, S. H., Winslow, N. S., Hoyt, H. H., Pritchard, W. R., and Nerlinger, C. M., *Cornell Vet.*, 1947, **37**, 324.

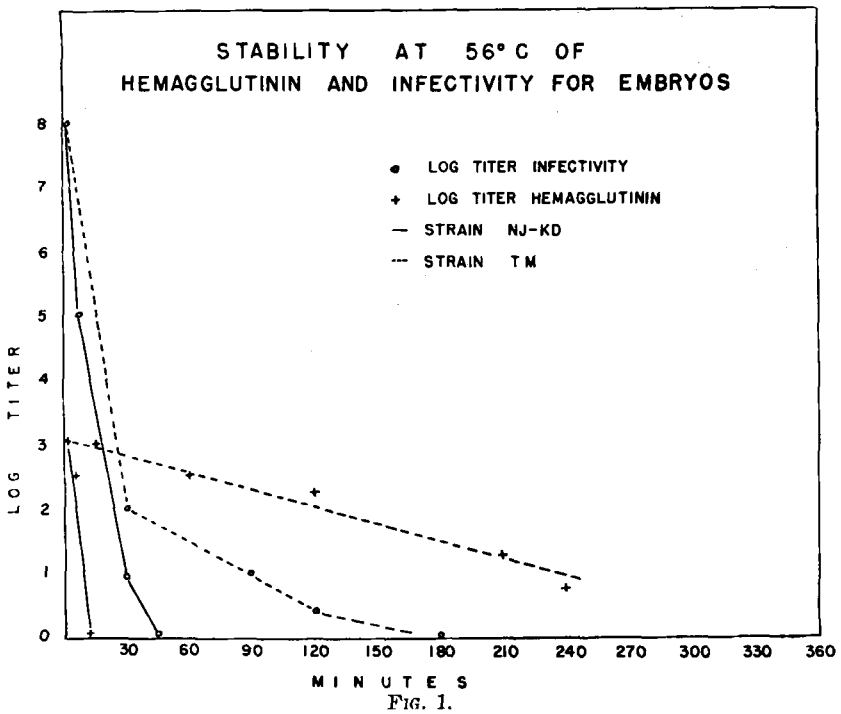

FIG. 1.

The stability of the hemagglutinins is depicted in Table II. Six periods at geometrically increasing intervals, beginning with 15 minutes and ending with 240 minutes, define the stability range of the hemagglutinins of the virus strains observed. The titer of all strains before treatment was approximately 1-1280, which is the usual titer obtained with red blood cells from selected fowls utilized in this study. Three of the 24 strains lost the power to agglutinate red cells within 15 minutes of heat treatment. Of these, FRB and HHT56 had low titers and NJ-KD no titer at all at 10 minutes. At 5 minutes, however, NJ-KD had a significant titer. Six of the 24 strains showed no hemagglutinins at 30 minutes heating. By 60 minutes the number of strains failing to agglutinate erythrocytes increased to 11 and after 2 hours heating to 13. Four hours of heating destroyed the agglutinin of all but 10 of the 24 strains. Four strains, KL, Ber, Cle and M, persisted for 6 hours. No agglutinins have ever been observed after 8 hours of heating at 56°C.

Immediately the question is asked: Is the embryo infectivity (EI) inactivated at the same rate as the hemagglutinin? Fig. 1 presents exploratory data that can be explained only by assuming distinct inactivation rates.

Discussion. Great variation in the stability of the hemagglutinin of individual Newcastle virus strains is apparent from the data given in Table II. Additional differences can be best shown by certain comparisons with studies made on influenza virus strains. Lauffer[5] presented evidence that the site of inactivation of the hemagglutinin and the infectivity for embryos was quite distinct. That Newcastle virus hemagglutinin and infectivity for embryos are inactivated independently is shown in Fig 1. Significant is the fact that demonstrable hemagglutinin of the NJ-KD strain is destroyed before demonstrable infectivity for embryos is lost. The infectivity of the TM strain, on the contrary, is inactivated more rapidly than the hemagglutinin. Most strains having hemagglutinins of low heat stability resemble NJ-KD in their HA/EI stability and most strains having hemagglutinins of high stability resemble TM.[15] Apparently, infectivity of the virus

[15] Upton, E., 1948. Thesis submitted for degree of Master of Science, University of Wisconsin.

for embryos has a narrower range of heat stability than the virus hemagglutinin. The former ranged from 30 to 180 minutes, as compared to 5 to 360 minutes for the latter. The descending order of susceptibility to heat treatment obtained by the Henles for certain activities of influenza virus is not paralleled with Newcastle virus. With the latter either activity, the HA or the EI, may be destroyed first.

The ability of the virus to fix complement in the presence of immune serum is very stable under heat treatment, withstanding 100°C for at least an hour. Using only three strains, 0.01% formalin failed to increase the stability of the hemagglutinin. The possibility still remains that certain other Newcastle virus strains, like some of the influenza strains, may have hemagglutinins more stable to heat in the presence of traces of formalin.

Comparisons of heat resistance of the hemagglutinating activity of the strain with the locality from which it was isolated suggests that a survey, based on many strains, may show a close correlation between them, especially if the time of isolation is considered. All five strains obtained from the eastern seaboard of the United States were of low heat resistance, 4 having either very low or no stability at 15 minutes. All 8 Canadian strains were highly heat resistant, 7 of them having hemagglutinins persisting for 2 to 4 hours. The strains from the Middle West showed more variation in their stability. When the time of isolation of these strains is taken into consideration, it is noted that of 3 obtained in 1946, all were of medium resistance; of 2 obtained in 1947, 1 was of medium and 1 of high resistance; and of 3 obtained in 1948, 1 was of low and 2 were of high resistance. It is possible that a shift in the heat resistance of the midwest strains is occurring and that it might be related to mutation of existing strains or to the introduction of new strains. The existence of such a possibility merits further investigation by this and other methods of strain characterization.

Conclusion. The stability of the hemagglutinin of different Newcastle disease virus strains, when subjected to increased temperatures, has been found to vary over a wide range. The stability of the hemagglutinin of 1 isolate was destroyed at 56°C in a period as short as 5 minutes, and that of another was diminished only after 6 hours exposure. The stability of the hemagglutinin of Newcastle virus is discussed in relationship to influenza viruses. A relation of heat stability of strains to place and time of isolation is observed.

16900

Comparative Nutritive Value of Butter and Vegetable Fats Under Conditions of Low Environmental Temperature.*

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It has been generally accepted by nutritionists that, aside from differences in vitamin potencies, animal and vegetable fats have essentially the same nutritive value. On diets containing an adequate amount of B

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