TABLE I.
Effect of APF, B_{12}, and B_{12b} on the Pig.

Lot No.	Ration fed	Avg starting wt, lb	Avg final wt, lb	Avg daily gain, lb
1	Basal	19.1	49.1	0.75
2	" + B_{12} injected*	19.4	45.8	0.66
3	" + " " +APF†	20.0	66.0	1.15
4	" + " orally‡	20.1	49.3	0.73
5	" + APF†	18.9	68.9	1.25

* B_{12} conc. which contained approximately equal parts of a relatively pure mixture of vit. B_{12} and B_{12b} (had at least 10 mg of B_{12} activity per gram of solids) obtained from Dr. T. H. Jukes, Lederle Laboratories, Pearl River, N.Y. B_{12} mixture was inj. peritoneally at the rate of 5 γ per kilo body wt once weekly.

† Lederle APF supplement (lot No. 8108-109) fed at a level of 1000 g per 100 lb of feed.

‡ The B_{12} and B_{12b} mixture was added to the feed at a level of 20 γ per lb of feed.

pigs fed a ration similar to that used in lots 2 and 4. Since APF contains aureomycin, and the aureomycin alone prevents the diarrhea, it is probable that aureomycin itself is responsible for preventing the diarrhea obtained with the pigs in this trial. The pigs fed APF had much smoother hair coats and showed more bloom than the pigs in the other lots where APF was not included.

The pigs fed APF (lots 3 and 5) gained at a considerably faster rate than the pigs fed the basal ration with or without B_{12} and B_{12b}. These data show that APF contains a factor(s) other than B_{12} or B_{12b} which is of importance for the pig. Jukes *et al.*(2) showed that aureomycin was of considerable benefit in increasing the rate of gain of pigs when added to a corn-peanut meal ration similar to that used in this trial. Therefore, aureomycin accounted for at least a part of the growth promoting activity of the APF used in this trial since it contained aureomycin.

Summary. A mixture of B_{12} and B_{12b} was of no benefit for growth when administered by injection or orally to the pig during a 40-day period under the conditions of this experiment. APF prevented a diarrhea which occurred periodically with the other pigs on experiment. APF fed pigs exhibited a smoother hair coat and more bloom. Further evidence was obtained to show that APF contains a factor(s) other than B_{12} or B_{12b}.

Received March 30, 1950. P.S.E.B.M., 1950, v74.

Agglutination of Mammalian Erythrocytes by Newcastle Disease Virus.* (17845)

NANCY S. WINSLOW, R. P. HANSON, ELIZABETH UPTON, AND C. A. BRANDLY.
From the Departments of Veterinary Science and Agricultural Bacteriology, University of Wisconsin, Madison, Wis.

The ability of Newcastle disease virus (NDV) to cause the agglutination of mammalian erythrocytes was studied as part of the problem of characterizing strains of the virus. Burnet(1), Clark and Nagler(2), Brandly *et al.*(3), and Chu(4) had reported

* These studies were made possible, in part, by funds derived through a special grant from the Bureau of Animal Industry, U. S. Department of Agriculture, and from support provided by public Laws 733 (9b1, 2 & 3).

Supported in part by funds from Project 711, Wisconsin Agricultural Experiment Station.

Published with approval of the Director of the Wisconsin Agricultural Experiment Station.

1. Burnet, F. M., *Aust. J. Exp. Biol. and Med. Sci.*, 1942, v20, 81.

2. Clark, Ellen and Nagler, F. P. O., *Aust. J. Exp. Biol. and Med. Sci.*, 1943, v21, 103.

agglutination of certain mammalian cells by NDV. Different strains of the virus were used by each of these investigators and since some of their findings were in disagreement, the possibility of the strain being the source of such differences was suggested.

In our studies, 31 strains of NDV isolated from natural outbreaks over a period of 15 years in this country, Canada, England and Italy were examined. Erythrocytes of 11 mammalian species were tested including those of man, guinea pig, mouse, fox, dog, horse, bison, cow, goat, sheep and elk.

Materials. Virus strains were selected from among those received by the NDV repository at the University of Wisconsin. Most of the strains had undergone from 2 to 8 egg passages and 6 strains were available after various egg passages ranging up to 60 passages. Individual histories and certain characters of these strains, *i.e.*, chicken pathogenicity and heat tolerance, were described by Hanson(5). For this paper the trisymbol designation of each strain, *i.e.*, KD-NJ-1945 indicating its code, place and time of origin will serve to identify the strain and its history. A prefix (T) or (H) signifies that the strain was obtained from a turkey or human source, and if not so specified the strain was derived from a chicken. Mammalian red blood cells were collected in 4.0% sodium citrate, centrifuged and washed 3 times in physiological saline. In all but one case cells were used within 3 to 5 days after drawing. A 1% suspension of cells in saline was used for the hemagglutination test. In most instances the agglutinability of blood cell samples from 5 or more individuals was determined for each species of animal. When possible the individuals were chosen to include divergent types of a species. Human blood samples included O, A and B blood types. Cattle blood was obtained from the Holstein, Guernsey, Jersey and Brown Swiss breeds. Some of the tests were made with cattle blood of known cell antigen types, supplied by Dr. Clyde Stormont, Department of Genetics, University of Wisconsin and some with cells obtained at various times during the estrous cycle. Cells of Toggenberg and Saanen breeds of goats and Suffolk sheep were used. Guinea pig blood was drawn from commercial stock pigs; mouse blood from strain H white mice originally from Rockefeller Institute stock and from the inbred mouse strain Z obtained from Iowa State College, Ames, Iowa. Silver foxes, mongrel dogs and Belgian horses supplied the blood from those species. Blood of bison and elk *Cervus canadensis nelsoni* came from the Wichita Mountains Wildlife Refuge, Cache, Okla., and was furnished by Dr. M. R. Irwin, Department of Genetics, University of Wisconsin. The bison blood was in two instances several weeks old at the time of testing.

Methods. The procedure of testing for hemagglutination activity (HA) was simple. The virus was used in the form of clear allanto-amnionic fluid pools from 4 to 5 13-day-old infected embryonating eggs. A simplification of the Salk hemagglutination technique was followed using only 2 dilutions of the virus, 1:10 and 1:20. To a volume of 0.5 ml of the diluted virus was added 0.25 m of the desired red blood cell suspension, and the test incubated at room temperature, about 25°C, for 30 to 45 minutes. Agglutination was determined by the pattern of sedimentation on the bottom of the tube. Suitable controls were set up with each test, *i.e.*, red blood cell, normal allantoic and normal amnionic fluids.

Results. Red blood cells from all tested individuals of 5 mammalian species, man, guinea pig, mouse, dog and bison, were agglutinated by all strains of the virus. On the other hand all samples of fox erythrocytes failed to be agglutinated by any strain of virus. The results of variably susceptible species are presented in Table I. Five strains of virus, KD-NJ-1944, 46967-Mich-1946, Lan-Wis-1947, 11914-Calif-1944, Herts-Eng-1933, did not agglutinate any of these cells. Cells from the 5 mammalian species were agglutinated by some strains of virus, not

3. Brandly, C. A., Moses, H. E., Jungherr, E. L. and Jones, E. Elizabeth, *Am. J. Vet. Res.*, 1946, v7, 289.

4. Chu, C. M., *J. Hyg.*, 1948, v46, 239.

5. Hanson, R. P., Thesis submitted for Ph. D. degree, University of Wisconsin Library, 1949.

TABLE I.
Hemagglutinating Activity of 21 Strains of Newcastle Disease Virus for 5 Species of Mammals.

	Mammalian erythrocytes*				
	Horse	Cow	Goat	Sheep	Elk
4F-Mass-1945	—	(—)	(—)	—	—
Levine-NY-1945	(+)	—	++	—	+
(T)Mayer-NY-1945	(—)	—	—	—	++
Roakin-NJ-1946	—	++	++	+	—
23-Iowa-1946	—	++	++	++	+
(T)547-Minn-1947	—	—	(—)	—	—
31747-Mo-1947	—	—	(+)	—	—
L-Kans-1948	(++)	—	(+)	—	++
M-Kans-1948	++	—	+	—	++
(T)Henry-Wis-1948	—	—	++	—	+
CG179-Calif-1944	—	—	—	—	++
670-Utah-1948	—	—	+		
Wat-Ont-1948	++	++	++	++	+
Ber-Ont-1948	++	—	++	++	++
(T)Peer-Sask-1948	(++)	++	++	+	+
All-Que-1948	(—)	—	(+)	—	++
Cle-Ont-1948	++	++	++	++	++
Lab-Ont-1948	—	(++)	++	++	++
Herr-Ont-1948	++	(++)	++	++	++
McVey-Ont-1948	++	(++)	++	++	++
M-Italy-1945	(++)	(++)	++	++	

* At least 5 individuals of each species.
+, ++, Degree of agglutination.
—, Lack of agglutination.
( ), Variation in agglutination, majority of individuals giving reaction noted.

TABLE II.
Agglutination by NDV of the Erythrocytes of 8 Species of Mammals.

	Investigator				
Mammal	Burnet*	Clark*	Brandly*	Chu*	Wisconsin†
Man	+	+	+	+	100
Guinea pig	+	+	+	+	100
Mouse	+			+	100
Dog			+	+	100
Horse	0	0	0	0§	54‡
Cow		+	+‡	0§	32‡
Goat	+	+		0§	50‡
Sheep		+	0	0§	40

* Expressed as agglutinated, +, or not agglutinated, 0.
† Expressed as % of virus strains demonstrating agglutination of the erythrocytes.
‡ Differences observed between samples of red blood cells from different donors.
§ Agglutinated only when purified virus was used.

agglutinated by others and inconsistently agglutinated by a few.

Comparative tests of 25 strains of the virus with horse, cow, goat and sheep cells showed that 6 strains agglutinated none of the 4 species, 13 agglutinated 1 to 3, and 6 agglutinated all species. Of the 25 strains of virus 19 agglutinated goat cells, 13 agglutinated horse cells, 10 agglutinated cow cells and 10 agglutinated sheep cells.

Table II summarizes selected data from the work of previous investigators and compares it with our results The ability of NDV to agglutinate certain mammalian red blood cells appears to be a distinct strain character

In addition to the strain of virus other factors influence the interaction of virus and red blood cells and must be considered in the evaluation of the effect of strains. Observations were made of the effect of variability of red blood cells among a given species, the effect of the pH of the reactants, and the

effect of egg passage upon the strain individuality.

Brandly(3) reported that the cells of some cows were agglutinated while those of others gave negative results. Consequently all our tests were set up to give a limited survey of cell susceptibility among individuals by testing 5 or more individuals of each species. Only 3 species of mammals, horse, cow and goat, yielded blood cells which differed in their agglutinability when tested by a single strain of the virus. Of 17 strains agglutinating horse erythrocytes 9 of these strains agglutinated only those of certain individuals. Four of 10 strains which agglutinated cow red blood cells agglutinated only certain samples and of 22 strains which agglutinated goat red blood cells 6 failed to clump the cells of all individuals.

The differential susceptibility of blood cells of individual cows was examined in some detail. It appeared to be unrelated to certain cell antigens, to the stage of estrus during which it was drawn, to lactation, or to breed. Samples of red blood cells obtained at diverse intervals from an individual cow yielded essentially the same agglutination spectrum.

The effect of pH upon the agglutination of red blood cells was investigated. In studying influenza virus Magill(6) had observed this to be a factor. The pH of the standard reactants in the HA test as determined with a Coleman potentiometer was 7.8. Preparations of Sorensen's phosphate buffered saline of pH values ranging from 5.6 to 8.2 were used as diluent for the test. The pH of the medium employed influenced the susceptibility of the erythrocytes to agglutination by NDV. The number of strains agglutinating cow erythrocytes increased from 35 to 82% when the pH was reduced from 7.8 to 6. While certain qualitative differences in agglutination were observed when the pH of goat erythrocyte suspensions was altered, the number of agglutinating strains remained about 70%. Sheep red blood cells, like those of the cow, were much more susceptible to agglutination at pH 6, 66% of the strains agglutinating at that pH as compared to 28% at pH 7.8. The effect of temperatures above or below approximately 25°C on hemagglutination was not ascertained.

Most of the strains were tested after only 3 to 6 egg passages. Two strains, Hertfordshire-Eng-1933 and KD-NJ-1945, available at both the 5th and 55th passage and at 5th and the 60th passage levels, respectively, showed no significant change in agglutinability occurring as a consequence of egg passage. After 6 passages strains 23-Iowa-1945, Milano-Italy-1945, and Leavenworth-Kans-1948 also showed no differences in their agglutinative activity.

Summary and conclusions. The ability of NDV to induce agglutination of mammalian erythrocytes of certain species is a character possessed by some but not all strains of the virus. The erythrocytes of certain individuals of such species as the cow, the horse, and the sheep are more susceptible than those of others to agglutination by NDV. Agglutination of mammalian red blood cells, as a rule, may be recorded accurately as the per cent of individuals whose cells are susceptible to the action of a given strain of NDV and not as a uniformly negative or positive result. This percentage of erythrocyte susceptible individuals may range from zero in some species to 100% in others but in most species lies between these extremes so that its consideration is warranted in every instance. It was demonstrated that the pH may affect the agglutination of mammalian red blood cells and, consequently, alter the interpretation of the test. If suitable controls of the variables of the test are included and the source of red blood cells is properly selected many strains of NDV may be differentiated by their mammalian agglutination range or spectrum.

The mechanism underlying these observations has not been investigated. The most obvious explanation predicates differences in the agglutinative activity of the virus strains, possibly associated with variable levels of enzyme production or activity. Chu would hold accountable differences in the levels of one or more inhibitors of hemagglutination. In either case, the activity or lack of activity

6. Magill, T. P. and Sugg, J. V., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 89.

seems to be associated with lines or strains of virus. The differing hemagglutinative susceptibility of individual donors suggests that the mechanisms involved are rather complex.

The phenomenon of agglutination of mammalian erythrocytes has practical application in maintaining the identity of stock strains of virus and possibly also in determining probable intermingling of the viruses of live vaccines and wild strains in field outbreaks of Newcastle disease.

Received March 27, 1950. P.S.E.B.M., 1950 v74.

Effect of Mercurials on Kidney Adenosine Triphosphatase Activity.* (17846)

ANDRES GOTH, JAMES HOLMAN, AND VIRGINIA O'DELL.

From the Department of Physiology and Pharmacology, Southwestern Medical School, Dallas, Texas.

Mercurial diuretics are capable of producing renal tubular damage. Preliminary experiments(1) have shown that kidney adenosine triphosphatase (ATP-ase) activity is inhibited by these compounds. Since very little is known about the nature of nephrotoxic action of drugs, it seemed of interest to study the possible relationship between the inhibitory effect of mercurials on kidney ATP-ase activity and the renal tubular injury produced by these drugs.

Material and methods. White rats of both sexes weighing approximately 200 g were used in this study. They were sacrificed by a blow on the head. The kidneys were homogenized at ice-water temperature in an all glass homogenizer. ATP-ase activity was determined by the method of DuBois and Potter(2). All enzyme determinations were done in quadruplicate. Inorganic phosphate was determined by the method of Fiske and Subbarow(3) using a Coleman spectrophotometer. ATP was obtained as the barium salt from the Nutritional Biochemicals Corporation, Cleveland, Ohio. The solution of the sodium salt was prepared just prior to the performance of the experiment. The mersalyl used was Salyrgan-Theophylline-Winthrop. The amount of theophylline in this preparation was found not to affect ATP-ase activity in the concentrations employed. In the *in vivo* experiments, one kidney of each rat was homogenized; the other was fixed in 10% formalin for microscopic studies. The ATP-ase activity of normal rat kidneys was determined simultaneously with the determinations done on kidneys of injected animals.

Results. Table I shows the effect of the addition of mersalyl and mercuric chloride on the ATP-ase activity of homogenized rat kidneys. Inhibition was observed consistently following the addition of these compounds in concentrations as low as 0.0001 M. The inorganic mercurial produced a greater inhibition at any given concentration than the organic compound.

TABLE I.
Effect of Mercurials on ATP-ase Activity of Homogenized Rat Kidneys.

	ATP-ase activity* per mg of kidney Final concentration of added drug		
Drug	0	0.0001M	0.00001M
Mersalyl	57	51	57
	58	50	55
Mercuric Chloride	57	35	54
	58	38	52

* μg of phosphorus liberated per hr.

* We are grateful to Dr. E. E. Muirhead for carrying out the microscopic studies

1. Goth, A., Holman, J., and O'Dell, V., *Fed. Proc.*, 1950, in press.

2. DuBois, K. P., and Potter, V. R., *J. Biol. Chem.*, 1943, v150, 185.

3. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.