a longer period, three doses of 0.4 units of insulin were injected at three-hour intervals. The averaged results in four animals are presented in Table 2.

Blood specimens obtained 90 minutes after the third injection of insulin show hypoglycemia, reduction of acid phosphatase to 25 per cent of normal, and reduction of the alkaline enzyme to within normal limits. Serum inorganic phosphorus is reduced.

TABLE 2

EFFECT OF THREE DOSES OF 0.4 UNITS OF INSULIN AT THREE-HOUR INTERVALS ON BLOOD SUGAR, SERUM PHOSPHATASES, AND INORGANIC PHOSPHORUS IN ALLOXAN-DIABETIC RATS

Time after injection of insulin	Blood sugar (mg./100 ml.)	Serum alkaline phosphatase (units/100 ml.)	Serum acid phosphatase (units/100 ml.)	Serum inorganic phosphorus (mg./100 ml.)
0 hrs.	425	384	3.9	11.8
1st dose				
1½ hrs.	150	346	3.1	11.1
3 "	154	284	2.3	11.5
2nd dose				
1½ hrs.	77	236	2.0	11.5
3 "	101	197	2.4	9.2
3rd dose				
1½ hrs.	51	161	1.0	10.2
3 "	122	132	1.0	9.8
9 "	410	206	2.3	7.4

Our findings indicate that the development of alloxan diabetes in rats is accompanied by an increase in serum alkaline phosphatase activity and that the administration of insulin produces a decrease in the activity of both the acid and alkaline enzymes. The initial decline in phosphatase activity following the injection of alloxan simulates that produced by the injection of insulin and is attributed to the release of insulin stores in the pancreas. When this supply is exhausted, alkaline phosphatase activity increases and remains elevated, the increase being parallel to the elevation in blood sugar. Both are reduced, again in parallel fashion, by the administration of insulin.

We have been unable to demonstrate any great increase in serum inorganic phosphorus following the administration of alloxan, although nearly all the values found were in excess of the preinjection level, and a few were greatly increased. Following the administration of insulin in the alloxan diabetic animal, reduction in the level of inorganic phosphorus was demonstrated. These findings are in accord with the well-known effect of insulin both in the diabetic and normal organisms (*1, 2*) and suggest that these alterations reflect changes in phosphatase activity.

References

1. BOLLIGER, A., and HARTMAN, F. W. *J. biol. Chem.*, 1925, **64**, 91.
2. CHAIKOFF, I. L., MACLEOD, J. J. R., and MARKOWITZ, J. *Amer. J. Physiol.*, 1925, **74**, 36.
3. GOULD, B. S., and SCHWACHMAN, H. *J. biol. Chem.*, 1943, **151**, 439.
4. JACOBS, H. R. *Proc. Soc. exp. Biol. Med.*, 1937-38, **37**, 407.
5. REINECKE, R. M. *J. biol. Chem.*, 1942, **143**, 351.
6. SHINOWARA, G. Y., JONES, L. M., and REINHART, H. L. *J. biol. Chem.*, 1942, **142**, 921.

The pH Stability of Viruses of Newcastle Disease and Fowl Plague[1]

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The recognition (*2*) of the immunologic identity of the causative agent of avian pneumoencephalitis with that of Newcastle disease revealed the existence of a serious threat to the poultry industry of the United States. The clinical features of the American form of the disease have allayed suspicion of its identity with the classical, hitherto highly virulent Newcastle disease (*1*). The differences between the American and the classical forms may result from the noted marked pneumo- and neurotropism of the virus isolated in this country and the pronounced enterotropism of a European strain of the virus (*7*). In Europe and elsewhere, Newcastle disease has been mistaken for possible forms of fowl plague and vice versa, and immunologic evidence has been obtained to support both dissimilarity (*5*) and similarity (*8*) of these diseases. However, recent immunologic and other comparative studies have provided strong support for the unity of each disease (*4*). The importance of these two diseases, the one a real, and the other a potential, threat to the poultry industry of this country, prompted studies to obtain further information on the relationship of the American and European Newcastle disease viruses and of these, in turn, to the virus of fowl plague.

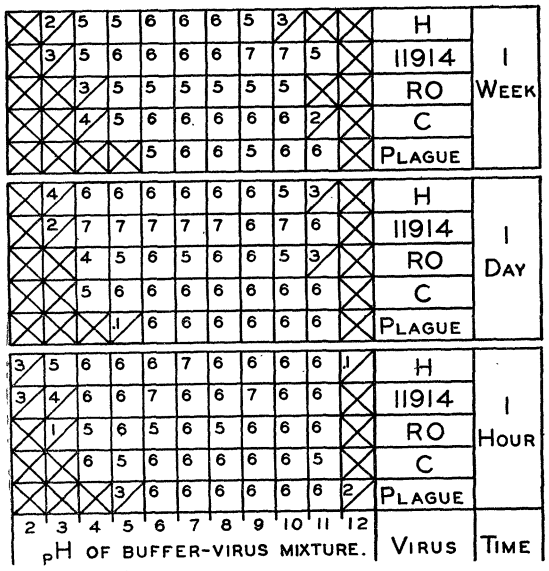
An evaluation by Doyle (*5*) of the effect of marked acidity and alkalinity on the Newcastle virus infectivity indicated greater resistance to the H- than to the OH-ions. The results of Pyl (*10*) in the case of the fowl plague virus showed that maximal stability of the infectivity occurred between pH 6.3 and 9.1 and that the virus was destroyed immediately at pH 4 and 12.7. This pattern was similar for brain and blood virus of chickens infected with the Brescia and Bologna strains of plague virus. These results and those for other viruses (*6, 10*) suggested or showed rather distinctive pH stability patterns.

Evaluations of the pH stability were made on four strains of the Newcastle disease virus, including the Hertfordshire strain (H) of English origin and three strains (11914, RO, and C) isolated from cases of "pneumoencephalitis" in California and provided by J. R. Beach, and on the Dutch East Indies strain of the fowl plague virus. A variant virus (Strain 4395), isolated from the plague virus in the course of other procedures (*9*), was subjected to similar study. The source of the virus used in all tests was the allanto-amnionic fluid of embryonated chicken eggs which had been infected with the respective viruses after 10–12 days of incubation. These materials were admixed in the ratio of 1:99, or, in a few tests, 1:9, with portions of a buffer solution (*3*) having pH values of from 2 to

[1] This work was done as a part of a research project conducted under the direction of a War Department Commission consisting of: Brig. Gen. R. A. Kelser, U. S. Army; B. E. Dyer, U. S. Public Health Service; H. W. Schoening, Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture; and E. B. Fred, University of Wisconsin.

12; the buffer solutions differed by a gradient of 1 pH and were prepared by the addition of NaOH, HCl, or distilled water so that the final concentrations of buffer salts were the same in all. These mixtures or the dilutions thereof in 1 per cent peptone broth (pH 7.8) were held at 2°–6° C. until used to test for the presence of virus. Infective virus was detected by its activity in 10- to 12-day embryonating chicken eggs following intra-allantoic inoculation. Tests for the virus were conducted at intervals of one hour, one day, and one week following the preparation of the buffer-virus mixtures. The pH values of the mixtures were determined by a glass electrode potentiometer after intervals of one day and one week.

The typical reproducible results obtained with the four strains of the Newcastle disease virus and the fowl plague virus are shown in Fig. 1. These results pertain to virus-buffer



VIRUS NONINFECTIVE.

INFECTIVITY TITER OF VIRUS REDUCED TO THE GIVEN DILUTION INDICATED BY RECIPROCAL OF LOG.

INFECTIVITY TITER OF VIRUS NOT SIGNIFICANTLY REDUCED; INFECTIVITY TITER INDICATED BY RECIPROCAL OF LOG OF THE DILUTION LETHAL FOR EMBRYOS.

FIG. 1

mixtures that were made in the ratio of 1:99. It will be noted that definite variations occurred in stability among the strains of Newcastle virus. In the acid range, two of the California strains (RO and C) showed comparable stability patterns, while one strain of this source was somewhat more resistant to acidity and was comparable to the strain (H) of English origin. All four strains were essentially similar in the presence of alkali after exposure for one hour, while infective virus of two of the strains (English and RO) diminished in concentration after one day, and still further variations were demonstrated after one week. A summary consideration of all four strains shows that after one hour a maximal stability existed over the range of approximately pH 4 to pH 11, while survival was possible over the range of pH 2 to pH 12; that after one week a maximal stability could be expected within at least the scale from approximately pH 5 to pH 9; and that survival of the virus should be anticipated approximately from pH 2 to pH 11. These stability ranges were considerably broader than that demonstrable for the virus of fowl plague.

The plague virus was much more sensitive to an acidic environment than the Newcastle virus, but was not distinctly unlike it with respect to alkali. The plague virus survived in maximal titer, after one week, from pH 6 to pH 11; the same maximal stability was demonstrated after exposure to the various pH conditions for one hour, but at this time infective virus was still present at pH 5 and at pH 12.

The pH values of the virus-buffer mixtures made in the ratio of 1:99 were found to drift toward the pH of the virus material (pH 7.8–8), but after one week this did not exceed 0.25 of one pH value in the extreme of the acid range or 0.5 of one pH value in the extreme of the alkaline range.

The results of a comparative study of the pH stability of the plague virus with that of a variant virus isolated from it are shown in Fig. 2. The ratio of quantity of virus material

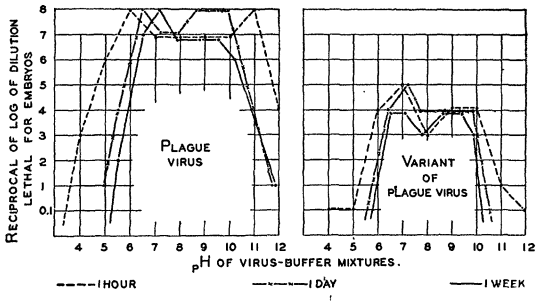


FIG. 2

to that of buffer employed here was 1:9. The stability patterns of the infectivity of both of these viruses were essentially similar; the differences that occurred between them are attributed to the lower concentration of the variant strain of virus that was attainable and/or to the ability to demonstrate it in small amounts by the route that was employed to inoculate the embryonated eggs. The differences between this test with the stock or typical strain of plague virus and the results reported heretofore appear accountable to the drift in pH and possibly the other factors associated with the use in the test of 10 per cent virus-infected allanto-amnionic fluid in the virus-buffer mixtures.

These observations are believed to reveal a close relationship among four strains of Newcastle disease virus, a distinct diversity between the virus of Newcastle disease and that of fowl plague, and a similarity between the fowl plague virus and a variant virus derived from it. The pH-stability patterns of a representative strain of the virus of classical Newcastle disease and of strains of virus from cases of so-called pneumoencephalitis in California are believed to confirm the immunological evidences of the identity of the causal agents of these two diseases which possess divergent tropisms. Likewise, the patterns obtained for a classical strain of the virus of fowl

plague confirmed information on its immunological identity with a variant virus of low virulence isolated from it. The results obtained with the fowl plague virus are in agreement with those recorded by Pyl. The stability patterns appear to be useful in the identification of the virus of Newcastle disease and of fowl plague.

References

1. BEACH, J. R. *Proc. 46th annu. Meet. U. S. Live Stock San. Ass.*, 1942' 203–223.
2. BEACH, J. R. *Science*, 1944, **100**, 361-362; BRANDLY, C. A., MOSES, H. E., and JONES, E. E. Special Report, Huntington Laboratory, January 20, 1944; Interim Report No. 4, Huntington Laboratory to War Research Service, March 27, 1944.
3. BEST, R. J., and SAMUELS, G. *Ann. appl. Biol.*, 1938, **25**, 509-537.
4. BRANDLY, C. A., MOSES, H. E., JONES, E. E., and JUNGHERR, E. L. *Amer. J. vet. Res.*, 1946, **7**, 289-306.
5. DOYLE, T. M. *J. comp. Path. Therap.*, 1927, **46**, 144-169.
6. FINKLESTEIN, H., MARX, W., BEARD, D., and BEARD, J. W. *J. inf. Dis.*, 1940, **16**, 117-26; EATON, M. D. *J. Immunol.*, 1940, **39**, 43-55.
7. JUNGHERR, E. L., TYZZER, E. E., BRANDLY, C. A., JONES, E. E., and MOSES, H. E. *Amer. J. vet. Res.*, 1946, **7**, 250-288.
8. MANNINGER, R. *J. comp. Path. Therap.*, 1936, **49**, 279-283.
9. MOSES, H. E., BRANDLY, C. A., JONES, E. E., and JUNGHERR, E. L. (To be published.)
10. PYL, G. *Behringw. Mitt.*, 1938, **9**, 6-19.

Effect of Gamma Globulin on Circulating Human Complement

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It has been shown by Davis, Kabat, Harris, and Moore (*1*) that the gamma globulin fraction of human serum separated by electrophoresis displays the same tendency to interfere with the activity of alexin as do globulin solutions prepared by other methods. These investigators noted that this effect was diminished or reversed by the addition of albumin and certain other serum proteins. However, since human gamma globulin is being used clinically for the prevention and modification of rubeola and infective hepatitis and has been under study as a therapeutic agent in scarlet fever, it appeared important to ascertain whether patients treated with this material exhibited any changes in the complement levels of their sera. This clinic has participated in a study of the therapy of scarlet fever with human gamma globulin, and the opportunity to study this question thus presented itself.

METHODS

Normal gamma globulin was furnished by Sharp and Dohme. This fraction was prepared under the direction of E. J. Cohn and his associates in the Department of Physical Chemistry, Harvard Medical School, from pooled human plasma collected by the American Red Cross. Only lots containing 45 or more units of streptococcal antierythrogenic antibody/ml. were employed. In the course of other investigations with this material it was found to be anticomplementary in the test tube even after the addition of considerable amounts of human albumin (*2*). As stated previously, in the hands of Davis and his colleagues, human albumin decreased or abolished the inactivating effect of globulin on alexin. It is probable, therefore, that the preparation administered in this study was anticomplementary in high titer. Nine patients, whose weight exceeded 60 pounds, received intramuscular injections of 60 ml. of the globulin, while one child weighing 50 pounds was given 50 ml.

Blood was obtained prior to the administration of globulin and at 2-, 4-, 8-, 12-, 16-, 20-, 24-, 36-, and 48-hour intervals thereafter. In each case, one specimen was omitted, withdrawal of blood being avoided in the hours after midnight. However, the schedule was so arranged that no interval was consistently neglected. The sera were separated immediately after bleeding and promptly frozen. Complement titration was carried out with sheep erythrocytes and rabbit amboceptor, 2 units of amboceptor being added to 0.5 ml. of 5 per cent sheep erythrocytes. Determinations were made in saline containing .01, .02, .03, .04, .05, .06, .07, .08, .09, .10, .20, .30, .40, and .50 ml. of the serum being tested, respectively. All tubes contained a total volume of 2.5 ml. and were incubated for one hour at 37°C. before reading. On the basis of rather extensive experience with this method it has been found that the alexin titer (50 per cent hemolysis) of human serum is usually 0.03–0.04 ml. It has been shown repeatedly with this technique that in certain diseases the complement titer may be depressed to 0.10 ml. or more (*5*).

RESULTS

The sera of the 10 patients examined before, and at frequent intervals for 48 hours after, the injection of normal gamma globulin all contained normal levels of complement; 0.04 ml. or less produced 50 per cent hemolysis regularly. Thus, no difference could be detected between control and postglobulin injection alexin titers.

DISCUSSION

Because of its high antibody content (*2*), human gamma globulin is being used as a prophylactic and therapeutic agent in certain diseases (*4*). In light of the known complement-binding character of this material, the sera of patients injected with globulin were examined for alexin levels. No change was demonstrated. In view of the albumin content of human serum, the dilution by the circulating blood, and possibly the relatively slow rate of absorption following intramuscular injection, it is not surprising that gamma globulin, in the dosages usually employed, produced no demonstrable alteration in complement activity when administered intramuscularly to man. If preparations suitable for intravenous injection become available, the possibility that they may produce complement depression when administered by this route should merit investigation.

References

1. DAVIS, B. D., KABAT, E. A., HARRIS, A., and MOORE, D. H. *J. Immunol.*, 1944, **49**, 223-233.
2. ENDERS, J. F. *J. clin. Invest.*, 1944, **23**, 510-530; also personal communication.
3. STOKES, J., JR., MARIS, E., and GELLIS, S. S. *J. clin. Invest.*, 1944, **23**, 531-540; ORDMAN, C. W., JENNINGS, C. G., and JANEWAY, C. A. *J. clin. Invest.*, 1944, **23**, 541-549; JANEWAY, C. A. *J. Amer. med. Ass.*, 1944, **126**, 678-680.
4. WEINSTEIN, L. Unpublished data.

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