amine was streaked on Whatman No. 3 MM paper and developed in 0.2*N* ammonia saturated with isobutanol according to the method of I. Gray and J. G. Young [*Clin. Chem.* 3, No. 4, 239 (1957)].

5. A portion of these data was presented at the 67th meeting of the Tennessee Academy of Science, 22 Nov. 1957. This work was supported in part by a grant from the National Heart Institute.

23 December 1957

Plaque Morphology and Pathogenicity of Vesicular Exanthema Virus

Two recent reports have described a correlation of plaque-type mutants of polioviruses with reduced virulence for monkeys and mice, respectively (*1*). However, variation of plaque morphology with antigenic type has not been described. This report describes intertype variations in plaque morphology among seven types of vesicular exanthema of swine virus, as well as intratype variations which have been correlated with differences in virulence for the natural host.

Plaque formation is obtained by infecting monolayers of a transmissible swine kidney cell, PK-2a (*2*), by means of the prescription bottle method of assay (*3*). The nutrient agar is similar to that described by Youngner (*4*). After incubation of infected cultures at 36° or 37°C, plaques can be seen as early as 24 hours. These continue to increase in size and number for 7 to 10 days, although 90 to 95 percent of the plaque count is obtained within 96 hours. Marked variation in plaque morphology is observed not only between virus types but also within virus types. Characteristic plaques of two of the seven virus types are shown in Fig. 1. With the exception of the G_{55} virus, two types of plaques are seen: a large, clear, round plaque which appears after 24 to 48 hours' incubation and increases in diameter to 5 to 8 mm within 96 hours, and a minute, opaque plaque, frequently irregular in shape, usually not visible until 72 to 96 hours have passed, which rarely exceeds 1.5 mm in diameter, even with 7 to 10 days' incubation. Pronounced differences in the ratio of the number of minute and large plaques, as well as in the size of plaques, are found between virus types. Some of the plaque characteristics observed are listed in Table 1.

The theoretical possibility that the large or minute plaques might be produced by a contaminating virus was excluded by the following series of experiments with E_{54} type virus. Stocks of "pure" large plaque formers (Lpf) and minute plaque formers (Mpf) of E type virus were prepared after three successive plaque purifications. The stocks could be considered pure only in a relative sense. As yet there is no suitable selective technique by which a small number of large plaque formers can be detected in the presence of a large number of minute plaque formers, and vice versa. Thus, viral suspensions which contained 1 large plaque former among 1000 minute plaque formers or 1 minute plaque former among 1000 large plaque formers were considered 99.9 percent pure. To determine whether both plaque variants were capable of producing vesicular exanthema, groups of swine were inoculated intradermally on the lip with 10^7 plaque-forming units of wild type (mixed) E_{54} virus, plaque purified E (Lpf) and purified E (Mpf). The ratios of minute plaque formers to large plaque formers in these three inocula were approximately 50:1, 0.001:1; and 1000:1, respectively. Temperatures were taken daily, and the presence or absence of primary and secondary vesicles was noted over an 18-day period. When possible, fresh vesicle coverings were obtained to determine the plaque type of the recovered virus. Two weeks after inoculation all animals were bled to obtain convalescent serum for neutralization tests.

Inoculation of the wild type virus and of the large plaque formers produced typical vesicular exanthema in four out of four animals, especially severe in the case of the latter. Four animals which received 10^7 plaque-forming units of minute plaque formers (which may have contained as many as 10^4 large plaque formers) exhibited no elevation of temperature over a 7-day period after infection, but each developed a single small lesion at the site of inoculation on the 6th or 7th day. No secondary lesions were observed. The absence of a febrile reaction prior to vesicle formation, the mildness of the primary lesions, and the absence of secondary vesicles constitute a picture of extreme atypical vesicular exanthema. These results indicate that the large plaque former is highly virulent for swine and that the minute plaque former either is essentially avirulent or is greatly reduced in pathogenicity.

Suspensions were made of vesicle coverings from infected swine and tested for plaque type of the recovered virus. Only the large plaque former was recovered, even from animals infected with the minute plaque former. This result substantiates the hypothesis that the large plaque former is the virulent virus particle and suggests that the mild disease produced in the swine inoculated with the minute plaque former was actually caused by the 0.1 percent of the large plaque former in the inoculum.

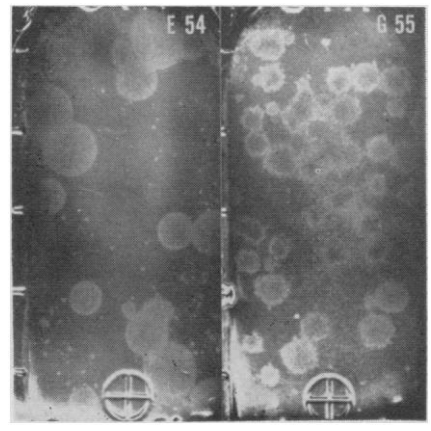

Fig. 1. Plaque morphology of vesicular exanthema of swine virus, types E_{54} and G_{55}, on transmissible swine kidney monolayers.

Cross-neutralization tests carried out by plaque assay method demonstrated the antigenic identity of the large plaque former and the minute plaque former. Convalescent sera from the three groups of infected swine were diluted 1 to 50, mixed with an equal volume of diluent containing about 10^8 plaque-forming units of either wild type E_{54} virus, E (Lpf) or E (Mpf), held 1 hour at room temperature, and assayed for residual infective virus. Serum from each group of infected swine neutralized 90 to 95 percent of the plaque-forming activity of all of the three virus suspensions, indicating close immunological relationship.

There appears to be no published report of the occurrence of such extreme differences in plaque morphology among different antigenic types of a single virus

Table 1. Characteristics of plaque variants of vesicular exanthema of swine virus on transmissible swine kidney cells.

Virus	Plaque size at 96 hr (mm)		Hour when 90% plaque count was obtained	No. of minute plaques per large plaque
	Large	Minute		
		Group I		
A_{48}	5–10	1	72	2
D_{53}	5–8	1	72	10
E_{54}	5–10	0.5	96	100
G_{55}	5–7		72	< 0.001
		Group II		
B_{51}	2–3	1	96	100
C_{52}	2–4	1	96	100
F_{55}	2–3	0.5	72	1–3

as those observed with vesicular exanthema of swine virus. Were the correlation only between plaque morphology and type, it would be interesting but not particularly meaningful in a biological sense. However, the fact that differences in plaque morphology of a virus were correlated with extremes of pathogenicity in a natural host lends more than academic interest to the observations. The findings with polio viruses previously mentioned and those with the virus described in this report (*5*) suggest that the correlation of physiological and morphological plaque variations with host pathogenicity may reflect a phenomenon common to other species.

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References and Notes

1. M. Vogt, R. Dulbecco, H. A. Wenner, *Virology* 4, 141 (1957); N. Takemori *et al.*, *Science* 126, 3279 (1957).
2. E. A. Stice, in preparation.
3. G. D. Hsiung and J. L. Melnick, *Virology* 1, 533 (1955).
4. J. S. Youngner, *J. Immunol.* 76 (1956).
5. This work was supported by the Office of Naval Research. Opinions contained in this report are not to be construed as reflecting the views of the Naval Service.

27 January 1958

Absence of Albuminlike Serum Proteins in Turtles

The differentiation of species of turtles of the genus *Pseudemys* by paper electrophoresis of serum proteins was reported recently (*1*). However, no turtle serum proteins comparable in electrophoretic behavior to human serum albumin were observed. Other workers (*2*) have reported marked differences between the electrophoretic patterns of turtles and snakes. A biochemical comparison of the total protein and albumin content of reptilian sera (*3*) revealed notably lower albumin values in turtles than in snakes. However, the particular salting out procedure of that study did not exclude alpha globulins from the albumin fraction.

Table 1. Rat, alligator, and turtle serum proteins (grams per 100 ml of serum). For this study, serum of one male specimen of Holtzman albino rat and of one male specimen of *Alligator mississipiensis* and pooled sera of three specimens of *Chelydra serpentina* were used. The "albumin" fraction from the serum of another specimen of *Chelydra* did give a faint biuret reaction.

Protein	Rat (g/100 ml)	Alligator (g/100 ml)	Turtle (g/100 ml)
Total protein	5.95	5.80	2.20
Albumin plus alpha globulins	3.12	1.80	2.03
Albumin	2.14	0.70	0.00
Alpha globulins	0.98	1.10	2.03
Other globulins	2.83	4.00	0.17

During our studies (*4*) with serum proteins of human beings and lower vertebrates with neoplasia, paper electrophoresis techniques did not reveal albuminlike components in sera of normal representatives of three major families of turtles. Turtle families and species studied were Chelydridae (*Chelydra serpentina*), Dermochelidae (*Dermochelys coriacea*), and Testudinidae (*Clemmys insculpta* and *Testudo gigantea*).

Sera were collected from clotted samples of blood obtained by cardiac puncture. Our paper electrophoresis was done with a Spinco apparatus, at 5 ma constant current for 16 hours. Paper strips were stained with bromphenol blue and were photoscanned and analyzed by means of the Spinco-Analytrol instrument. Specimens of human serum were included in each run. Rat and alligator serum proteins were compared with those of a turtle by a biuret procedure following a modified salt-ether fractionation (*5*) of the blood sera.

The turtle sera examined appear free of a human-like albumin serum protein component, according to electrophoretic analyses (Fig. 1). Ether-salt fractionation and biuret analysis did not consistently reveal albuminlike protein in the serum of *Chelydra* (Table 1).

The findings are provocative from the viewpoints of comparative biochemistry, physiology, and systematics. Albumin synthesis is a function that has long been ascribed to the parenchymal cells of the liver (*6*). Such cells are reported to be structurally cirrhotic-like in the liver of fish, amphibians, and reptiles (*7*). Interestingly enough, paper electrophoresis of the blood serum of Elasmobranchii (*8*) has revealed no component with the mobility of albumin. Correlations between liver histology and protein biochemistry are not available for reptiles. Such studies might be of phylogenetic value. Boyden and Paulsen (*9*) have emphasized the value of electrophoretic studies of serum proteins as a step toward understanding the biochemical evolution of the vertebrates. However, physical chemical criteria, in addition to paper electrophoresis, and protein analyses of greater sensitivity than the biuret reaction are necessary before one can satisfactorily define the nature of the presence or absence of "albumin" in the sera of turtles or other lower vertebrates.

The results reported here suggest an absence of a human-like serum protein with electrophoretic properties of albumin in more than one genus and family of turtles. Similar independent observations of Zweig and Crenshaw (*1*) are supported by our work.

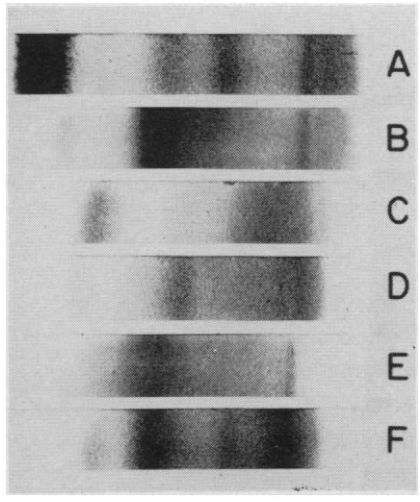

Fig. 1. Paper electrophoresis patterns of serum proteins of turtles and of the serum proteins of a human being (*A*) and an alligator (*B*) for comparison. Other patterns represent the turtle species *Dermochelys coriacea* (*C*), *Clemmys insculpta* (*D*), *Testudo gigantea* (*E*), and *Chelydra serpentina* (*F*).

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References and Notes

1. G. Zweig and J. W. Crenshaw, *Science* 126, 1065 (1957).
2. H. F. Deutsch and W. H. McShan, *J. Biol. Chem.* 180, 219 (1949); T. L. Gleason and F. Friedberg, *Physiol. Zoöl.* 26, 95 (1953); H. C. Dessauer and W. Fox, *Science* 124, 225 (1956).
3. E. Cohen, *Science* 119, 98 (1954).
4. For this work, paper electrophoresis facilities were kindly provided by Dr. Donald Pinkel, Roswell Park Memorial Institute. Acknowledgement is made of the technical assistance of Mr. P. C. Clark and Mr. K. Crampton. *Testudo* and *Dermochelys* sera were donated by the Serological Museum, Rutgers University.
5. W. Q. Wolfson *et al.*, *Am. J. Clin. Pathol.* 18, 723 (1948).
6. S. C. Madden and G. H. Whipple, *Physiol. Revs.* 20, 194 (1940).
7. H. Elias and H. Bengelsdorf, *Anat. Natshr.* 1, 273 (1951).
8. H. Irisawa and A. F. Irisawa, *Science* 120, 849 (1954).
9. A. A. Boyden and E. C. Paulsen, *Serol. Museum Bull.* 18, 7 (1957).
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13 December 1957

Inhibition of Ribonuclease by Polyacids

Heparin and other sulfated polysaccharides have been reported to act as competitive inhibitors of beef pancreas and rodent liver ribonucleases (*1, 2*). Two well-recognized effects of heparin have been reproduced with the polyacids