resistance than the normal rat. These hemodynamic changes should be considered in interpreting results of experiments in which such animals are used.

Summary. A technique was developed by means of which determinations of cardiac output, total peripheral resistance, mean circulation time, total blood volume and central blood volume can be repeated in a given rat. The above hemodynamic parameters were determined before and after ganglionic blockade with pentolinium in both male and female rats. Blood pressure fell approximately 50% after blockade; the responsibility for the reduction being equally divided between fall in cardiac output and reduced total peripheral resistance. Total blood volume was not significantly changed and central blood volume decreased slightly. Mean circulation time was increased after blockade. There was no apparent difference between male and female rats in their response to blockade.

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A Hog Cholera Virus-Fluorescent Antibody System. Its Potential Use in Study of Embryonic Infection. (28455)

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A new concept in virus-host relationships was introduced by Gregg(1) who concluded that rubella virus which attacked the mother in the first third of gestation could adversely affect the developing embryo. Although many later reports have appeared to support the general virus concept of Gregg, they are also based largely on circumstantial evidence. Experimental approaches to production of malformed fetuses in animals have failed to clarify basic mechanisms of damage to embryonic and fetal tissues. Rhodes(2) and

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Blattner and Heys(3) have reviewed this subject.

Rabbit attenuated hog cholera (HC) viruses were introduced in the early 1950's to be used as vaccines to control HC. Young (4) associated stillborn and malformed swine fetuses to inoculation of the dams with attenuated HC viruses during the first 30 days of a 114 day gestation period. Experimental production of malformed fetuses included demonstration of HC virus in the spleens of the fetuses but not in the spleen of the dams(5). Specific areas of damage or mechanisms were not determined.

An HC virus-fluorescent antibody (FA) system has been developed as a tool to evaluate virus-host relationships which contribute to malformed embryos and fetuses. Since this HC virus-FA system also is useful in diagnosis of naturally occurring HC, this preliminary report is made so that laboratories associated with the National HC Eradication Program will be aware of a FA system to diagnose HC.

Materials and methods. Viruses. The hog cholera (HC) viruses used were the same as those described earlier(6) except for the virulent Ames strain, designated V_4, and the tissue culture (TC) viruses designated TC_1 and TC_2. V_4 HC virus was obtained from the Nat. Animal Disease Laboratory, Ames, Iowa. TC_1 HC virus is virulent and originated in this laboratory whereas TC_2 HC virus, an attenuated virus, was obtained from a commercial source.

Experimental pigs. Pigs used for preparation of anti-HC immune globulin and for virus assays in tissues were pathogen-free genetically small pigs(7) of hysterectomy origin(8). They were housed in modified Horsfall-Bauer isolation units(9) and isolation brooders(10). Pigs used to produce globulin were 6 weeks old when immunization procedures were initiated. Virus infected tissues were obtained from pigs inoculated intranasally-orally when 1 week old. Adult secondary pathogen-free pigs were used similarly to determine variance among age groups.

Fluorescent antibody. FA was prepared stepwise as follows: 1) Four pigs were injected intramuscularly with a rabbit origin modified live HC virus (R_1), without serum. Immunity of these pigs was challenged 10 days later by inoculation of 1000 MLD/50 V_1 HC virus. All pigs survived the challenge and 7 days later were given 150 ml of plasma intraperitoneally. This plasma was prepared from littermate pigs inoculated intramuscularly 6 days previously with V_2 HC virus. A mixture of 125 ml of plasma containing V_1 and V_3 HC viruses similarly prepared was given to each pig 7 days after the V_2 virus. The 4 pigs were exsanguinated 10 days later and serums were harvested.

2) Globulin was prepared from swine serum dialyzed against an ammonium sulfate solution at 4°C with stirring. The final concentration of ammonium sulfate in the serum and surrounding solution was 1.39 M. The resulting precipitate, which contained primarily gamma globulin, was dialyzed *vs* several changes of 0.05 M sodium carbonate-bicarbonate, pH 9.0, until the solution was free of sulfate.

3) Twenty-four mg of crystalline fluorescein isothiocyanate (FITC)† in 4 ml of 0.05 M carbonate-bicarbonate, pH 9.0, were dialyzed into 50 ml of a 2% solution of the above globulin with stirring for 24 hours at 4°C to effect conjugation. The labeled globulin was then dialyzed *vs* 0.01 M sodium phosphate pH 7.5, containing 20 g of AG 2 — X4 anion exchanger (chloride form)‡ to remove excess FITC, for 24 hours, and then against the same buffer without the exchanger for 24 hours.

4) Chromatography of labeled globulins on diethylaminoethyl cellulose (DEAE cellulose)§ was performed essentially as given by Goldstein *et al.*(11,12). Absorbancy of the fractions at 280 mμ and 495 mμ was measured on a Beckman model DB spectrophotometer. Protein concentrations were determined by the method of Lowry *et al.*(13). Fluorescein-protein ratios were calculated as given by Goldstein *et al.*(11).

HC virus-FA interaction. The finished

† Lot No. 1036, Sylvania Chemical Co., Orange, N. J.

‡ Purified Dowex, California Corp. for Biochemical Res., Los Angeles 63, Calif.

§ Type 20, Brown Co., Boston, Mass.

TABLE I. Preferential Ratios in Fluorescent Antibody-Hog Cholera Virus Interactions.

Tagged globulin fraction	Fluorescein protein × 10^3	Fluorescence
Sample No.	Range	
4-237 I	3.8-5.4	Acceptable
4-237 II	6.7-9.3	Nonspecific
4-247 I	3.2-5.3	Acceptable
4-247 II	6.4-8.4	Nonspecific

conjugate was adjusted to a protein concentration of 0.1 mg/ml in 0.1 M phosphate buffered saline at pH 7.5. One ml of conjugate was mixed with 0.1 ml of guinea pig complement‖ and the mixture was applied to 4 μ cryostat¶ sections of normal and HC infected swine tissues per Coons and Kaplan (14). Complement was added to enhance antigen-antibody (A-AB) union. Stained sections were examined for specific fluorescence with a microscope equipped with a dark field condenser and an ultra-violet light source.**

Results. Preliminary trials using tissues of V_1 HC infected pigs stained with FA prepared against $V_{1, 2, 3,}$ HC viruses indicated that the enhancing substance(s) in guinea pig complement were essential in the A-AB union. Without this enhancement, fluorescent staining was weak or absent. Globulins passed through the DEAE-cellulose columns also showed different intensities of activity as indicated in Table I. The lower ratio fractions were used on subsequent exploratory evaluations.

Since the globulin prepared had a heterologous origin, *i.e.*, viruses R_1, V_1, V_2, and V_3, served as antigenic components, tissues from pigs inoculated with different strains of HC virus were stained with FA and examined.

The results presented in Table II indicate an A-AB reaction with all of the virulent viruses used to prepare the hyperimmune serum. Adult pigs reacted the same as young pigs in all instances. Fuorescence was also demonstrable with the Ames strain (V_4) of virulent virus which was not an antigenic component in preparation of the FA. The R_1 virus, which was used to immunize the serum pigs before challenge and hyperimmunization, was not detectable as a tissue antigen in infected pigs.††

HC antigens were detectable in tissues of infected pigs as early as 3 days and up through 10 days. Fluorescence was less intense in pigs evaluated on the third day and in pigs evaluated 8-10 days. Tests were not made on pigs beyond 10 days. Viral antigens were stained in tissue cells within brain, spleen, kidney (Fig. 1), lymph node, lung and parotid salivary gland (Fig. 2).

All pigs in which fluorescence was found showed evidence of viral antigen in the spleen and lymph nodes. Both reticulo-endothelial and lymphoid tissues were involved. Fluorescence in the kidney was limited to the proximal and distal convoluted tubules and to the collecting tubules. Kidney tissues were not always stained. Cells in those areas stained by FA also showed pyknosis and

TABLE II. Intensity of Fluorescent Antibody Reaction in Swine Tissues Infected with Different Hog Cholera Viruses.

Hog cholera antigen*	No. pigs inoculated	Intensity of fluorescence†
Virulent viruses		
V_1	12	++
V_2	6	+
V_3	2	++
V_4	3	+
Modified viruses		
Porcine: P_1	2	—
Rabbit: R_1	8	—
R_2	2	—
Tissue culture viruses		
TC_1 (virulent)	35	++
TC_2 (attenuated)	2	—

* Viral antigens in frozen sections from organs of HC infected pigs.

† FA composed of globulins of pigs injected with R_1 and V_1 HC viruses and hyperimmunized with V_2 and a V_1-V_3 combination of HC viruses.

++ Intense, + Acceptable, — None.

†† An exception was 1 pig inoculated with R_1 virus. He became ill at 9 days and died 11 days later. Histopathological lesions were typical of HC.

‖ Difco Laboratories, Detroit, Mich. Lyophilized product reconstituted to recommended volume of 2 ml.

¶ Lipshaw "Cryotome" Model 1500 Cryostat, Model 50 AB Microtome (rustless). Lipshaw Mfg. Co., Detroit, Mich.

** Leitz Ortholux Microscope equipped with an HBO-200 L-2 light source, 2 mm UGI exciter filter and Euphos barrier filter. E. Leitz, Inc., New York.

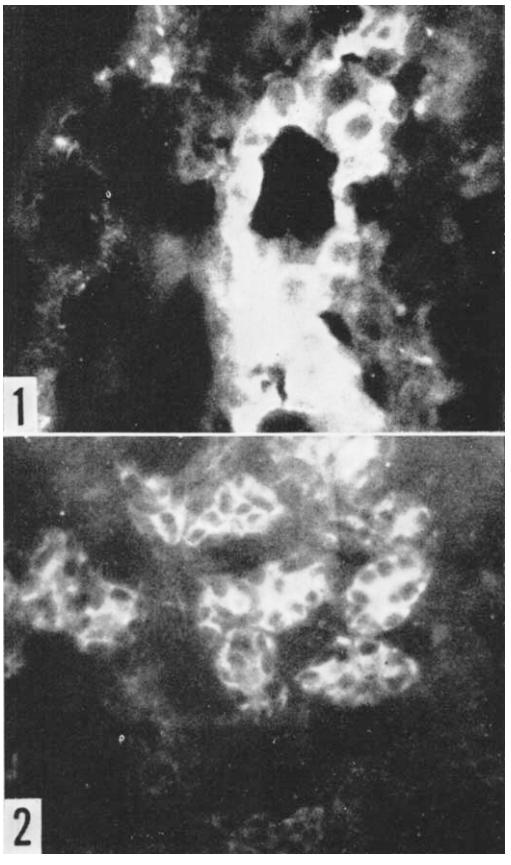

FIG. 1. Fluorescent antibody staining of V_1 hog cholera antigen in swine kidney tubule cells. Pig killed 7 days postinoculation. Mag. 375 ×.

FIG. 2. Fluorescent antibody staining of V_1 hog cholera antigen in the acini of swine parotid salivary gland. Pig killed 7 days postinoculation. Mag. 375 ×.

karyorrhexis in sections stained with hematoxylin and eosin. Staining of the acini cells in parotid salivary glands was more consistent and general than in the kidneys. The epithelial cells lining the ducts of these glands apparently contained no HC antigen as they failed to stain. Fluorescence was variably observed in brain but involved vascular tissues and cells within the spaces of Virchow-Robin when present. Although lung tissue was not consistently stained, when staining did occur it was intense with involvement of the epithelial lining of the bronchii and bronchioles. Some sub-epithelial mucous glands were stained.

The fluorescence observed was considered to be specific. Tissues from normal pathogen-free pigs showed only background blue-gray autofluorescence. Inhibition of staining was complete when infected tissues were treated with untagged anti-HC globulin prior to treatment with FITC-anti-HC globulin conjugate. A similar FITC-globulin preparation specific for pseudorabies failed to stain HC infected tissues. Pigs infected with pseudorabies, virus pneumonia of pigs, exudative epidermitis and swine influenza failed to accept HC FITC-globulin for a positive FA test.

Discussion. Experimental approaches have failed to define the location of HC virus in infected embryonic tissues. The FA technique herein described should allow for precise localization of the viral antigen in embryonic and fetal tissues. Often it is difficult to isolate or identify the virus. In the experiments of Young *et al.*(5), virus was detected in the spleens of fetuses but not in the spleens of the dams. Since HC is characterized by persistent viremia, any organ of the infected fetuses connected with the blood-vascular system would be expected to yield virus. One obvious lesion in fetuses damaged by modified HC virus was pitting of the kidneys. Associated with these lesions was accumulation of fluids in the pleural and peritoneal cavities with anasarca of fetal tissues. Malfunction of the kidney tubules could explain accumulation of these fluids. Deposits of HC antigen in kidney tubule cells (Fig. 1) stained with FA lends plausibility to this theory. Viral antigens in the lymph nodes may indicate a destruction of these tissues with a resulting lymphatic blockage, ascites and generalized edema. Experiments are under way with swine embryos and fetuses from dams infected with TC_1 strain of HC in an attempt to observe HC antigen in specific fetal tissues.

Caution in interpretation is essential in FA staining of HC viral antigens in swine tissues. An affinity for FA by white blood cells in the spleen and lymph nodes may be confusing. This is overcome by observation of all fluorescent areas in these tissues by both ultraviolet and incandescent light under dark field. True FA stained cells will not be apparent under incandescent light.

Fluorescence must be in the cytoplasm or nucleus of individual cells to be a valid indicator of HC viral antigen.

Enhancing factor(s) in the guinea pig complement aided union of A-AB to give a visible FA reaction. A similar requirement was indicated by Liu *et al.*(15) for a FA test for primary atypical pneumonia.

This preliminary report is presented primarily because the U. S. Dept. of Agriculture is embarking on an HC eradication program. Present methods for specific identification of HC involve inoculation of groups of susceptible and immune swine. The method is expensive, and tests take up to 10 days for tentative evaluation. The FA method reported by Dunne(16) is indirect since it involves inoculation of TC with HC virus several days before the FA test. The FA method herein described takes less than 2 hours to accomplish the same purpose. Obvious refinements need to be made to provide diagnostic procedures adequate for routine use.

Summary. A hog cholera virus-fluorescent antibody system has been developed using pathogen-free pigs as the source of globulins. Heterologous globulin was developed by hyperimmunizing pathogen-free genetically small pigs with 3 strains of virulent hog cholera virus. Success of the system appeared to be based on use of pigs having minimum of non-specific globulins, complement for antigen-antibody union, and globulins which approach maximum levels of conjugation to fluorescein isothiocyanate. Use of this system in the study of viral infections of swine embryos and for rapid diagnosis of hog cholera are discussed.

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Influence of Alpha-Ketoacids on the Respiration of Brain *in vitro*.* (28456)

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Several inherited defects in amino acid metabolism are characterized by mental retardation and central nervous system dysfunction, as well as by elevated plasma and urine concentrations of particular amino acids or amino acid metabolites(1). These include phenylpyruvic oligophrenia(2), citrullinuria(3), hyperprolinemia(4), hydroxypro-

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