and nuclear fractions of the liver tumor. The microsomes from each tissue contained only small amounts of the vitamin and coenzyme. The finding that these tumors contain low levels of coenzyme A is in harmony with the observation of Potter and his associates that homogenates of such tumors contain low amounts of the enzymes that oxidize oxalacetic acid.

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A Complement Fixation Test for Equine Virus Abortion.* (18233)

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(Introduced by Roy C. Avery)

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Equine virus abortion (E.V.A.) was first described as a clinical entity, of unknown etiology, by Dimock and Edwards(1). Subsequent reports(2-8) established the viral etiology, and more fully characterized this disease. E.V.A. is an epizootic contagious disease of Equidae characterized by abortion, usually in the late stages of pregnancy, without signs of infection of the mare before or after parturition. The most striking pathological lesion noted in the aborted fetus are small foci of necrosis in the liver. Other changes worthy of note are hemorrhages in the abdominal and thoracic organs, edema and congestion of the lungs, and pleural effusion. Necrosis and intranuclear inclusions are the chief histopathological features. E.V.A. has been reported(6,9) from 12 states in this country, and five foreign countries. Although data regarding the incidence of this disease in the United States are incomplete, the majority of cases appear to occur in central Kentucky, principally on thoroughbred farms. abortion rates ranging from 10% to 85% in exposed groups of animals indicate the extreme degree of contagiousness of the virus. Little, however, is known concerning the mode of transmission of E.V.A.; apparently it is not venereal, since the stallion has not as yet been implicated. The disease has been induced artificially by feeding pregnant mares unfiltered infected placenta or fetal tissue, and by the injection of bacteria-free filtrates of infected tissue. The incubation period of such infected animals is from 18-30 days.

The host range of the virus appears to be narrowly limited. Small success has attended the efforts of those who have attempted to grow this virus in animals other than the natural host. Dimock, Edwards, and Bruner (6) were unable to demonstrate infection in mice, rabbits, guinea pigs and chick embryos. Serial passage through guinea pig foetuses(10) has failed to maintain the virus. Histopathological evidence of infection in newborn Syrian hamsters has been adduced by Goodpasture and Anderson(11,12). Other investigators (9,10) have not succeeded in demonstrating

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6. Dimock, W. W., Edwards, P. R., and Bruner, D. W., *Ky. Agric. Exp. Sta.*, 1942, Bull. 426.

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8. Dimock, W. W., Edwards, P. R., and Bruner, D. W., *Ky. Agric. Exp. Sta.*, 1947, Bull. 509, 4.

9. Bruner, D. W., Doll, E. R., and Hull, F. E., *The Blood Horse*, 1949, v58, 31.

10. Doll, E. R., Personal communication.

11. Anderson, K., and Goodpasture, E. W., *Am. J. Path.*, 1942, v18, 555.

infection in day-old hamsters. There is therefore no method by which the presence of the virus of E.V.A. can be unequivocally demonstrated save by the injection of the presumptively infected material into a pregnant mare, with resulting abortion of a foal showing the characteristic gross and microscopic pathological changes. Such a test is impracticable, save for a very few laboratories. (It may be pointed out, in passing, that injection of pregnant mares with infected fetal tissue is by no means followed by abortion in all cases.) A simple, convenient, rapid and unequivocal test for detecting the presence of this virus would be highly desirable. From the standpoint of the rather considerable economic loss sustained by breeders of thoroughbred horses, a test which would give information on the presence of antibodies might be of great value in the diagnosis and prognosis of E.V.A., and help in evaluating the efficacy of prophylactic vaccines. A specific serological test should meet all these requirements. Since the complement-fixation test has been successfully used in other virus infections, it seemed preferable to attempt to devise such a test for E.V.A. This paper constitutes a report on the complement-fixation test developed in these laboratories for the detection and measurement of the specific antigen and antibody of E.V.A.

Methods and results. Preparation of antigens. Since the lungs and liver, and to a lesser extent the spleen, of aborted foals, have been found to be the most heavily infected organs, these organs were used as potential sources of antigen. The frozen tissue (which had been preserved at −18°C for from 2 to 9 months) was cut into blocks averaging about 1 cm on a side, and the blocks washed in repeated changes of ice cold physiological saline until no further trace of hemoglobin could be obtained. After overnight storage at 4°C in saline, the washed tissue blocks were drained, blotted nearly free of moisture, weighed, refrozen at −18°C, ground to a smooth paste, and diluted 1:2 with cold saline on the basis of the weight before refreezing. Low-speed centrifugation (3000 r.p.m. for 10 minutes at 4°C) was found, in early experiments, to yield an antigen which appeared to be unsatisfactory for complement-fixation tests. The ground tissues were therefore centrifuged at 13,500 r.p.m. for 30 minutes at 4°C, and a crystal-clear hemoglobin-tinged supernatant obtained. This supernatant, without further treatment, was tubed in convenient small amounts, and stored at −18°C until used. Organs from normal, uninfected animals were treated in the same manner, for use as one control of antigen specificity in the complement-fixation test.

Preparation of sera. All blood specimens used in the tests were drawn with the usual aseptic precautions, allowed to clot; the expressed serum pipetted off, clarified by centrifugation, and stored in small ampoules at −18°C until use. Potentially positive sera were available for testing from horses which had acquired E.V.A. naturally, and from horses in which this disease had been artificially induced. An attempt was also made to obtain positive serum by artificial immunization of two male horses. The first horse, a yearling stallion, was given an injection into each testicle of 3 ml of a 1:2 dilution of equal parts of ground lung, liver and spleen antigen. (It may be noted here that when the testicles were removed ten days after the injection, no specific lesions could be demonstrated microscopically.) The second horse, a 2-year-old gelding, was given a single intravenous injection of 15 ml of a mixture composed of the supernatants from both low- and high-speed centrifugation of ground infected lung tissue. For serum controls, blood was obtained from normal stallions, and from normal mares with no history of E.V.A.

The complement-fixation test. Throughout all experiments we have used the 50% hemolysis end-point complement-fixation test, following the spectrophotometric method of measurement developed by Mayer *et al.*(13), and Kent *et al.*(14). A veronal-buffered sa-

12. Goodpasture, E. W., and Anderson, K., *Am. J. Path.*, 1942, v18, 563.

13. Mayer, M. M., Eaton, B. B., and Heidelberger, M., *J. Immunol.*, 1946, v53, 31.

14. Kent, J. F., Bukantz, S. C., and Rein, C. R., *J. Immunol.*, 1946, v53, 37.

line solution(15) was used for all dilutions. In the qualitative test, a mixture of 1 p. antigen, 1 p. serum (1:5 dilution), and 1 p. complement (containing 3 H_{50} units), is allowed to incubate at 4°C for 18 hours. At the end of this period of primary incubation, 1 p. of optimally sensitized sheep erythrocytes is added to the system, followed by a second period of incubation for 45 minutes in a 37°C water-bath. Adequate controls are included in each test. Sera showing 80% hemolysis or less, and which are not anticomplementary, are considered positive, and subsequently titrated in two-fold serial dilution.

The first trial run, using as antigen the supernatant from low-speed centrifugation of a heavily infected liver, gave a negative result with serum from a proven case of naturally acquired E.V.A. Predicating the presence of antibodies in the serum, and fixation of complement by these antibodies in combination with adequate amounts of antigen, it was considered that this negative result might be a reflection of low viral content of the antigen, which had had to be diluted 1:160 to avoid non-specific fixation of complement. Accordingly, in all subsequent tests the supernatants of high-speed centrifugation of infected tissues, were employed as antigens. Four antigens were prepared by this method: liver, spleen, lung No. 1, and lung No. 2. (Each antigen was derived from a different infected fetus. Tests for non-specific fixation of complement, in the presence of 1 H_{50} unit of complement, showed that the strongest concentrations in which these antigens could be used were 1:80, 1:640, 1:40, and 1:100, respectively. These antigens, in the stated dilutions, were then set up against the serum used in the first run, and against serum obtained from the stallion which had been injected intratesticularly 10 days before bleeding. Complete fixation of the 3 H_{50} units of complement occurred with lung No. 2 antigen, with both test sera; lung No. 1 antigen showed slight but definite fixation with both sera; neither the liver nor the spleen preparations gave any fixation with either serum. None of the 4 antigens fixed complement in the presence of control serum obtained from the immunized stallion before intratesticular injection. A test to determine the optimal dilution of lung No. 2 antigen revealed strongest fixation at all dilutions of antiserum, in the highest non-anticomplementary concentration (1:100) which could be employed. Control antigen prepared from the lungs of a normal horse in precisely the same manner as the test antigen, and used in proper non-anticomplementary dilution, did not fix complement in the presence of either positive or negative sera.

Using lung No. 2 antigen it has been possible to demonstrate the presence of antibodies in the serum of seven horses with proven E.V.A., both naturally and artificially acquired, and in serum from the gelding immunized by the intravenous route. The titers of sera obtained from infected horses ranged from 1:16 to 1:320. Both of the sera obtained from the two immunized male horses showed strong fixation at 1:640. Serum from two non-immunized male horses, and from ten normal female horses with no history of E.V.A. have been consistently negative.

Conclusions and summary. The complement-fixation test described in this paper provides a means whereby the presence of antibodies to the virus of E.V.A. can be detected and quantitatively estimated in serum. The lung antigen employed in the test is specific in the sense that it does not react with sera from normal horses, and that similarly prepared antigen from the lungs of a normal horse does not react with positive sera. (Whether or not the antigen will react with antibodies to other viruses, and whether or not different strains of this virus exist, remain to be investigated.) By means of this test information may now be obtained on the immunological course of E.V.A. in diseased mares, which will perhaps help in the early diagnosis of E.V.A., and in the prognosis of this infection. The response of 2 horses to single injections of infected material has revealed the potent antigenicity of the prepara-

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tions. The possibilities of prophylactic immunizations, using the complement-fixation test to check the progress of the immunization, are obvious. Lastly, the test may be employed as a means of determining the presence or absence of the virus, whether in the natural host, experimentally infected animals, or inoculated tissue cultures. Experiments are in progress on an evaluation of the complement-fixation test, as employed for these purposes.

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A Cannula for Simultaneous Drainage of Both Cavae in Artificial Heart Experiments.* (18234)

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One of the many problems connected with a mechanical heart preparation is that of the cannulation of both the superior and inferior vena cavae. The superior vena cava may be cannulated easily through the azygous vein, which may then be ligated after removal of the cannula [Gibbon(1); Leeds, Gray, and Cook(2)]. The inferior vena cava may be cannulated through the right auricular appendage which is then ligated after removing the cannula [Gibbon(1)]. In order to simplify the cannulation, a single right angle cannula was constructed which permits blood from both the cavae to pass through it and thence flow to the pump. This procedure saves a step in the experiment, and appears to be more satisfactory than having two cannulas. It is certain to drain the entire caval flow and, therefore, should be preferable to the method of Jongbloed(3), who passes a catheter into each cava near the auricle and applies suction to draw the blood from the cavae to the pump. With the new cannula ligated in place no blood from the cavae can enter the right auricle, and it must all be diverted. The right auricle or ventricle may be opened with very little blood loss since only the coronary flow enters the operative site. The latter constitutes about 5 or 6% of the cardiac output [Harrison, Friedman and Resnick(4)].

The purpose of the present communication is to describe a right angle cannula which is passed through the azygous vein and which in one operation permits the drainage of

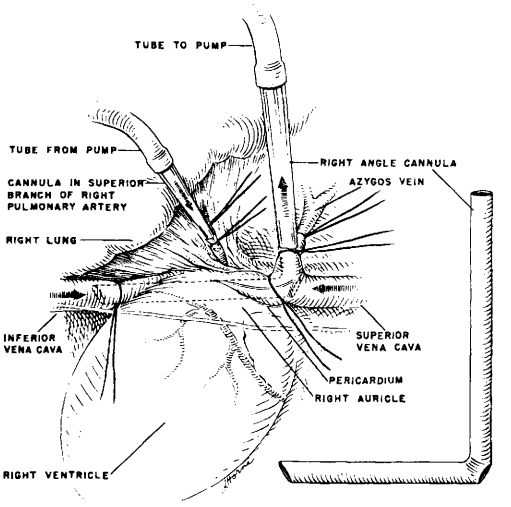

FIG. 1.

Diagram which shows cannula inserted through azygous vein into superior and inferior *vena cavae.* The ligatures prevent any caval blood from entering the right auricle. The cannula which returns the blood from the pump to a branch of the right pulmonary artery is also shown.

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2. Leeds, Sanford E., Gray, Norman, and Cook, Orrin S., *Surg., Gyn., and Obst.*, in press.

3. Jongbloed, J., *Surg., Gyn., and Obst.*, 1949, v89, 684.

4. Harrison, T. R., Friedman, B., and Resnick, H., *Arch. Int. Med.*, 1936, v57, 927.