writer examined serial sections of some 200 cat embryos of appropriate age in the Huntington Collection of Columbia University, and to his great surprise found a gall bladder in a 10.5 mm. embryo which was in the very act of disappearing. Indeed, the cystic duct had already atrophied, leaving the terminal vesicle isolated in the ventral body wall at a point just medial to the place where the right umbilical vein enters the liver. From this early embryonic position the gall bladder normally recedes. But in this particular embryo, withdrawal of the gall bladder had apparently been prevented by a circlet of veins which enclosed the neck of the organ, as a result of which the cystic duct would seem to have first thinned out and then ruptured. Two of these veins were of unusual interest since they exhibited an anomalous forking of the central end of the vitelline vein; so that instead of following its usual circuitous course to the liver through the portal mesentery, this vein had bifurcated, one limb of it anastomosing with the adjacent right umbilical vein and the other extending straight through the *septum transversum* to that portion of the left common cardinal vein which is destined to become the coronary sinus. Whether the actual rupture of the cystic duct was due to the pressure of these anomalous veins is, of course, a matter of conjecture. But at least it shows the very early period at which the gall bladder disappears and indicates that the method is not unlike that which normally occurs in the pigeon, namely, isolation and atrophy following rupture of the cystic duct.

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Observations Upon the Nature of the Virus of Hog Cholera.

CHARLES W. DUVAL.

From the Department of Pathology, College of Medicine, Tulane University

Although hog cholera has been the subject of extensive investigation by immunological methods, little has appeared in the literature concerning the nature of the specific virus, aside from the discovery of DeSchweinitz and Dorset[1] that the causal agent is filtrable through Berkefeldt and Chamberlain candles. This paper records certain observations made with the virus in the course of an experimental study.

The virus in the blood of hog cholera withstands rapid desiccation

[1] DeSchweinitz and Dorset, Cir. No. 41, Bureau of Animal Industry.

over sulphuric acid, unslacked lime or other hygroscopic agents *in vacuo* at 0° C., without its infectiousness and antigenic property being apparently affected. The resulting virus-powder may be kept in sealed glass containers for years and probably indefinitely without loss in viability, virulence and antigenicity. Though the virus in the powdered form keeps indefinitely, after it is redissolved in a suitable solvent such as normal physiological salt solution, it loses in viability and antigenic property at the same rate and under like conditions as is the case with the undesiccated virus-blood.

The desiccated virus-serum can be utilized in conjunction with immune serum (desiccated or fresh) to produce an active immunity in the hog. The virus-powder is first thoroughly dissolved in the fresh immune serum or in the dissolved desiccated immune serum in the proportion of one part virus to 2 parts serum. This *in vitro* admixture of virus and immune sera in no way prevents the production of an active immunization of the host following its subcutaneous injection, and gives rise to no objective symptoms of infection. The virus-powder and desiccated immune serum may be mixed and kept for an indefinite period in a sealed glass container, away from light and at 0° C., without appreciably affecting its antigenic power. These facts permit of the deduction that the virus is not destroyed *in vitro* through the action of a lytic immune body, nor is it apparently affected *in vivo* through the additional action of host complement. Repeated experiments with activated and inactivated virus-immune serum mixtures show conclusively that the immune body in hog cholera is antitoxic rather than lytic. Viability and virulence of the virus-powder are not affected when mixed with fresh immune serum. The mixture in which the immune serum is less in quantity than the virus, per mg. of weight, the animal injected invariably develops cholera. A larger quantity of immune serum than virus-serum is employed in making a mixture sufficiently potent to neutralize the toxin elaborated during the period in which the animal is acquiring an active immunity. That the virus is viable and multiplying in the injected hog is confirmed by the fact that the withdrawn blood is highly infectious.

It has been determined by animal experiment that the virus of hog cholera at no period during the infection is intracellular, in so far as the blood cells are concerned. Repeated injections into young non-immune hogs of large quantities of the freshly washed blood cells have failed to infect. Therefore, it is highly advantageous in working with the virus to eliminate the cellular elements of the blood and use only the serum since the cells are worthless from the stand-

point of virus. To obtain the cell-free virus-serum the freshly drawn blood of the infected hog is first defibrinated and then centrifugalized under sterile conditions, and the clear supernatant serum pipetted off.

By the method of desiccation, 1 cc. of virus-serum yields approximately 100 mg.; the dried defibrinated whole blood gives a residue of 156 mg.

While the virus content of infected serum varies depending upon a number of factors, as the size and age of the hog, duration and severity of the infection, commonly 1 cc. of cell-free virus-serum contains approximately 4000 fatal doses for the hog of 40 to 50 pounds weight. Expressed in terms of desiccated virus-serum the minimum lethal dose is 0.01 mg.

Our attempts to cultivate the hog cholera virus upon artificial media have met with only partial success. No growth of the virus occurs on ordinary laboratory media under any conditions. Excepting where semi-solid hog plasma medium was employed, prepared after the method of Noguchi,[2] the inoculated virus rarely survived longer than 3 weeks regardless of the temperature at which the cultures were maintained. Multiplication apparently takes place in the special hog plasma medium when kept under anaerobic conditions and at 40° C. While colonization of the virus was at no time observed in the plasma medium there was noted in the deeper parts a cloudiness similar to that produced by the artificially grown *Treponema pallidum*. Further proof that virus growth had occurred was shown by infection of the hog with the sixth subculture in amounts no greater than the original material used for culturing. It is unlikely that the virus simply remained viable and was carried over to subcultures in sufficient numbers to infect. Preparations made from the cultures and examined with the dark field show only innumerable minute globular bodies which are similar in morphology to those described by Flexner and Noguchi[3] for poliomyelitis. Nothing whatsoever was seen in the cultures that even resembled spirochaetal organisms. It is of interest to note that frequently *B. cholera suis* (T. Smith) grew out in pure culture from the desiccated virus-serum. In one instance a pure growth of the hog cholera bacillus was obtained from virus-powder more than 2 years old.

[2] Noguchi, H., *J. Exp. Med.*, 1919, xxx, 13.

[3] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.