Since the ovary is normal in every respect it seems likely that the fish was a female and that the abnormally shaped testis arose after the ovary was fully formed. Had the testis developed along with the ovary in the young fish, it might be expected that

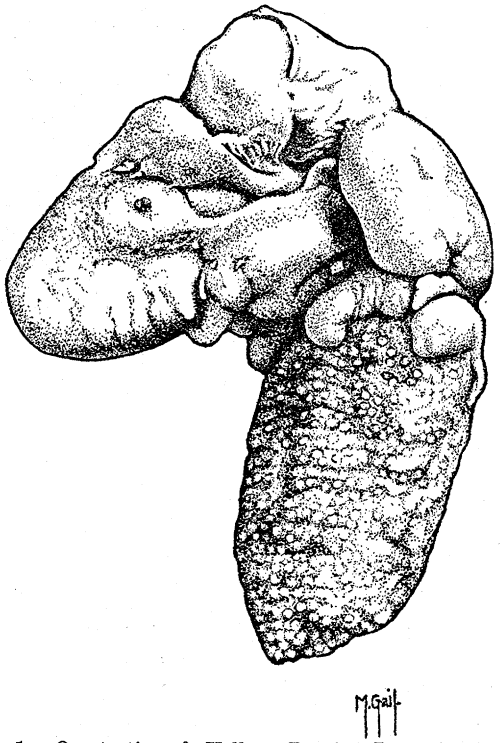

FIG. 1. Ovo-testis of Yellow Perch. Irregular testis above, normal ovary below.

neither ovary nor testis would be entirely normal in position or shape.

What stimulation might cause the production of a testis in the presence of a normal ovary it is hardly profitable to guess. It should be noted, however, that nothing in either portion of the gland is so antagonistic to the other as to prohibit the normal germ cell maturation in the other.

Nothing could be made out of the accessory sexual apparatus but if sperm and eggs were provided with a means of escape from the body, the case might be classified as a rare instance of functional (even if teratological) hermaphroditism.

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THE CULTIVATION OF VACCINE AND OTHER VIRUSES IN THE CHORIO-ALLANTOIC MEMBRANE OF CHICK EMBRYOS

THE successful infection of the chorio-allantoic membrane of chick embryos with fowl-pox virus led us to investigate the effect of the inoculation of other viruses upon this apparently highly susceptible tissue. Thus far it has been found that the viruses of vaccinia and herpes simplex infect the membrane notwithstanding the fact that vaccine is only slightly pathogenic for adult fowls, and that repeated attempts to infect adult and young chickens with the virus of herpes simplex by a variety of routes have failed. The susceptibility of the chick's chorio-allantoic membrane to infection with the virus of herpes indicates that the embryonic cells offer a more favorable condition for the growth of some viruses than adult chicken cells. This embryonic tissue would therefore seem to be a promising medium to test the infectiousness of material from those animal diseases which have been proven to be or are supposed to be caused by viruses, but have not yet been transplanted upon an alien host.

The great susceptibility of the chorio-allantoic membrane of chick embryos to infection with vaccine virus is shown by the readiness with which a vaccinal lesion develops and spreads on the membrane, and by the enormous multiplication of virus at the site of inoculation. Bacteria-free virus has now been carried through many generations by passage every three days through the membranes of embryos ranging in age from seven to fifteen days. The infected embryos rarely live longer than four days after inoculation because of the rapid spread of the lesion. At the end of three days a large lesion has developed and this stage has been arbitrarily chosen as preferable for transfer, and for histological and cytological study.

The technique of inoculation is the same as that recently described by Woodruff and Goodpasture,[1] except that we now outline the positions of the membrane in young embryos, 7 to 11 days old, by candling; so that we are sure the window will be cut directly over it. Also the surface of the egg shell is coated with a thin layer of melted paraffine over an area somewhat larger than the proposed window, in order to obviate infection from pieces of shell. After the shell is removed the shell membrane is also coated with paraffine of low melting point so that this membrane may be torn with a sharp pointed instrument on three sides of the window, then folded back and cut with scissors, thus exposing the chorio-allantoic membrane. In this way powdered and fragmented egg shell can not so readily fall upon the exposed serosa and contaminate it.

Bacteria-free vaccine virus obtained from the testis

[1] A. M. Woodruff and E. W. Goodpasture, *Amer. Jour. Path.*, 1931, 7, 209.

of a rabbit has been used to initiate the infection.[2] When transfers are to be made from infected membranes the cover-glass is removed from the window, and the upper portion of the membrane is exposed as widely as possible by breaking off surrounding egg shell with aseptic precautions. The infected membrane is then cut out and placed in a Petri dish containing sterile .9 per cent. saline solution. A small piece of tissue from the thickened and hemorrhagic area is removed with scissors and smears from this are stained by Morosow's method to determine the presence of Paschen bodies.[3] These bodies appear in enormous numbers in the infected membranes although they are difficult to demonstrate in smears from the skin and cornea of chickens infected with vaccinia. Their presence in the smear is diagnostic of infection. Other smears are stained with Loeffler's methylene blue to determine the presence of bacteria. If Paschen bodies are abundant and no bacteria are demonstrable a piece of infected membrane measuring from .5 to 1 mm in diameter is inoculated upon the membrane of each of five or six embryos. We have found twelve day embryos to be perhaps most satisfactory. Pieces of the remaining infected membrane are then inoculated into culture media and others are placed in 50 per cent. glycerol in .9 per cent. saline and in fixatives for histological study.

Sections of infected membranes fixed twenty-four hours in Zenker's solution and stained in a 2 per cent. solution of acid fuchsin for ten to thirty minutes, then counter-stained with Loeffler's methylene blue and differentiated in absolute alcohol, present a most instructive picture. Guarnieri bodies are found in all stages of development, finally filling and replacing almost completely the cytoplasm of the cell. It is to be observed that Guarnieri bodies occur not only in the epithelial cells of ectoderm and endoderm, but in great abundance, reaching a relatively large size, in capillary and venous endothelium, in fibroblasts and possibly in the smooth muscle cells of blood-vessels. It is to be noted that the inclusions develop in great numbers and to a large size before there is any necrosis or cellular infiltration, although inflammatory exudate is abundant in older necrotizing areas. The effect of the infection on capillary endothelium is of interest. The endothelial cells are frequently stimulated to an active multiplication, so as to form small nodules and cords completely obliterating the vascular lumens. Petechial hemorrhages particularly from capillaries are numerous. The ectodermal cells of the membrane are affected first and apparently most severely, although there is often a more widely spread effect upon the mesoderm. Entoderm is affected least.

Smears made from infected membrane frequently show masses of partially dispersed Paschen bodies within the cytoplasm of cells; and pieces of membrane examined in distilled water show round or oval masses in which minute bodies are to be seen in rapid Brownian motion, just like those seen in the inclusions of fowl-pox and *Molluscum contagiosum*. Studies are being made upon the relation of the Paschen bodies to the structure of Guarnieri bodies. The abundance of both these elements and the unusually large size of the latter make this tissue especially adaptable to such an investigation.

Virus passed through eight generations in chick embryos has been applied to the skin of two or three day old chicks after plucking the down. Macroscopic nodules about 1 mm in diameter appear at the site of inoculation within three days. Remaining apparently stationary for two or three days they rapidly recede. Chicks recovered from vaccinia were infected two weeks later with fowl-pox virus and the lesions evoluted exactly like those of fowl-pox in normal chicks. Vaccinia in the chick induces no immunity to fowl-pox.

Our observations suggest that the chick embryo might be used advantageously for cultivating bacteria-free vaccine on a large scale for human vaccination.

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[2] A strain of Levaditi neurovaccine was kindly supplied by Dr. T. M. Rivers.

[3] M. A. Morosow, *Centralbl. f. Bakteriol.*, Pt. I, Orig., 1926, 100, 385.

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