water, then dipped into a one half per cent. aqueous eosin (Grubler's *wasserlich*) for a half a minute and washed quickly again. (Washing is done in large volumes of water.) They are then stained in Weigert's aniline methyl violet made up as follows:

SOLUTION 1

Absolute alcohol	33 cc
Aniline oil	9 cc
Methyl violet in excess	

SOLUTION 2

Sat. aqueous solution of methyl violet
(For methyl violet use Grubler's 6B only)

One part of Solution 1 is used to nine parts of Solution 2 for the stain. The two solutions will keep separately, but after mixing, the stain will only last about ten days. It seems to be best about three to eight days after making.

The tissues are stained in the methyl violet about two hours, washed well in tap water and put into Lugol's solution for ten or fifteen minutes, after which they are again washed. Each slide is then blotted thoroughly with filter-paper and subsequently differentiated in a mixture of one part aniline oil to two parts xylol. After washing in several changes of xylol they are mounted in balsam.

The fibrin and gram positive organisms as well as ciliary basal bodies are stained a deep purple; the dark blue nuclei are well differentiated. All outlines of cells can be seen distinctly due to the counter-stain. Blood corpuscles are generally of a pale blue color, though at times they remain pink.

This method has been used particularly in the study of fibrin and of pneumococci (though other gram positive organisms have been stained as successfully) and has proved of special value in the determination of intracellular bacteria.

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SPECIAL ARTICLES

AN OVO-TESTIS IN THE YELLOW PERCH (PERCA FLAVESCENS)

HERMAPHRODITISM occurs fairly regularly in some genera of Teleost fishes (Serranus and Chrysophorous) and teratological hermaphroditism has been observed and described in the Burbot, Carp, Cod, Eel, Top Minnow (Fundulus, Lebistes, Xiphophorus), Stickleback, Herring, Ling, Perch (Perca fluviatilis), Salmon, Sargus, Shad, Whiting, Wrass, Sheepshead, Croaker, Trout and Loach. In the latter cases the testis and ovary are seldom both full sized and normal. Occasionally both gonads are developed to a point which would make functional hermaphroditism possible but usually one gonad is fully formed and normal while the other is partially developed or fragmentary and recognizable only by histological examination. Secondary sex characters such as colors, gonapods, internal structures and behavior are in some instances profoundly affected. In other instances no such modifications are present.

Non-functional hermaphroditism has been observed in the European Perch (Perca fluviatilis) by Halbertsma, Hoek, Ivanzov, Skogman and Chevey, but no record appears in the literature of any similar condition in the American Perch (Perca flavescens).

In the case described here the specimen was discovered by an employee of the Johnson Fish Company, of Green Bay, Wisconsin, in early February and the entire gonad was preserved in alcohol.

The ovarian portion of the gland is 52 mm long and appears to be normal in every respect. It is located in the normal position. An abundance of large ovocytes were plainly visible before dissection and on histological examination the usual arrangement within the ovary of fully formed ovocytes and of undeveloped germ cells was demonstrated. The stroma and tunica were also normal. The testicular mass is somewhat larger than that of a normal testis and is located anterior to the ovary and while it is immediately adjacent to the ovary, it is marked off sharply by texture and color. The testis is abnormal in position, therefore, and superficially appears to be tumorous and misshapen. Several large and many small lobes appear, none of which have the shape of the normal testis. Microscopic sections of the testis, however, show little that could be called abnormal. All lobes are filled with masses of spermatozoa. Each lobe is subdivided by connective tissue septa into cysts just as in the normal testis. At the peripheral margins of the lobes there are flat, lens-shaped cysts of large spermatogonia or primordial germ cells, a relationship which also exists in mature normal testes in February. The only unusual histological feature is the thickening of the walls between some of the lobules and between the testicular and ovarian portions of the gland.

Special attention was given to the region between the ovarian and testicular portions of the gland in order to discover any possible transitional zone. No transition occurs. On both testicular and ovarian sides of the connective tissue junction the line of demarkation is abrupt and there is no evidence of anything but normal tissue.

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

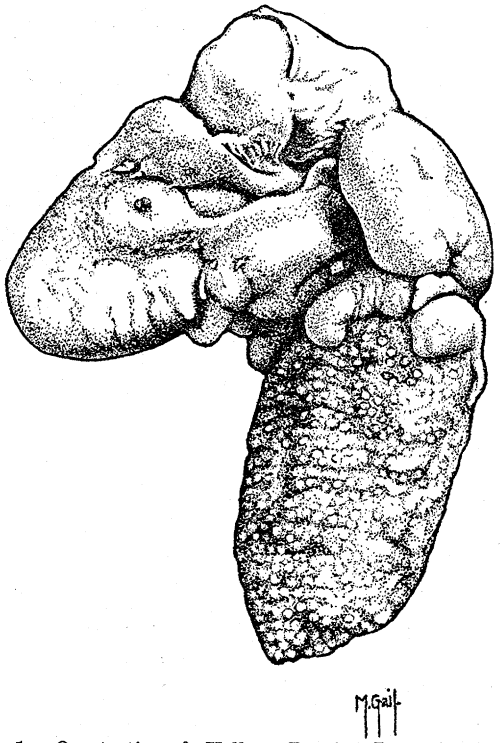

FIG. 1. Ovo-testis of Yellow Perch. Irregular testis above, normal ovary below.

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.