

supply in the earlier stages of growth may stunt the plant so that absorption in the later stages is much less than would occur with a normal plant.

D. R. HOAGLAND

DIVISION OF AGRICULTURAL CHEMISTRY,
UNIVERSITY OF CALIFORNIA

UNHEATED EGG-YOLK MEDIA

For some years the writer has been using unheated egg-yolk media and has found them especially valuable in studying one of the fowlbroods caused by an organism (*Bacillus larvæ*) which offers considerable difficulty in its cultivation. In a paper "Further Studies on American Fowlbrood" to be published in the *Journal of Agricultural Research* reference is made to the employment of such media successfully in the study of this species. Believing that the fact might be of interest to those studying diseases caused by organisms for the cultivation of which unheated animal products are being employed and possibly also to those using heated egg media in their work, the technic used in the preparation of these media is given at this time.

These are prepared by adding simply a sterile aqueous suspension of egg-yolk to the different media commonly used in the laboratory. The egg suspension is obtained as follows: After being disinfected the shell of the egg is broken the white poured off and the yolk dropped into a flask containing about 70 c.c. of sterile water. By agitating the flask a uniform suspension of the yolk material is obtained. This is then transferred to sterile tubes by pipetting, and stored until needed. On standing the suspension separates into a more or less translucent supernatant fluid and an opaque lighter yellow-colored sediment.

In preparing the egg media about 1 c.c. of the egg-yolk suspension is added to each 5 c.c. of the base medium. If only the supernatant fluid is used a clearer medium will result. Egg agar has been the most useful of these media in the work referred to. The base should be at least 1.5 per cent. agar and after being liquefied should be cooled to between 45° and 50° C. before the suspension is added.

Tubes may be inclined and stored until needed. The medium may be inoculated and plates made, or sterile plates may be poured. Although the pipetting of the sterile suspension rarely results in contamination of the media, if convenient to do so, it is well to test them for sterility after this step is taken. The egg suspension itself is a medium of some differential value.

Eggs known to be recently produced are preferable for the egg-yolk suspension, although those obtained from the market labelled "strictly fresh" have usually been satisfactory. The shell is disinfected conveniently by immersing the egg in a suitable solution for a few minutes. A 1:1,000 mercuric chloride one is satisfactory for the purpose. Alcohol and solutions of carbolic acid and formalin have been used but the latter two unless gloves are employed are unpleasant to the hands. After removing the egg from the solution, the shell is broken about one end and removed with forceps sterilized conveniently in the direct flame. The white being poured off the limiting membrane of the yolk is broken and the yolk material is poured into the flask containing the sterile water. The degree of transparency of the supernatant fluid depends somewhat upon the amount of water used in making the suspension. Occasionally contaminations are encountered. These are usually detected by changes in the appearance of the suspension following incubation.

G. F. WHITE

BUREAU OF ENTOMOLOGY,
WASHINGTON, D. C.

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