LABORATORY INCUBATOR FOR THE BIOLOGICAL STUDY OF CHICK EMBRYO

ONE of the very important problems in experimental embryology is the control of physical factors of incubation. Neither commercial nor laboratory incubators in use are adequately equipped or standardized. Consequently, the experimental data of different investigators[1] could hardly be compared and very often disagree. There is no uniformity whatsoever in comparative study of the initial growth, growth-cycles and some metabolic processes of chick embryo.

With a view to provide a perfect control of all physical factors of incubation (such as temperature, humidity, quality of air, ventilation and movement of eggs) in experimental work at the poultry husbandry department of Cornell University, a special electric laboratory incubator has been assembled and used successfully for almost two years. This incubator has facilities to serve: first, as a respiration apparatus, having both closed and open circuits; and secondly, as an ordinary incubator. Furthermore, it is so adapted that each variable physical factor can be independently and accurately controlled. The accuracy is shown by the average variations of incubation temperature and humidity, which, for instance, were less than 0.2° C. and 1.0 per cent. relative humidity. In addition to this, automatic alarms prevent accidents, and make it possible to do all the biological laboratory work without embarrassment or loss of time.

On the accompanying diagram there is shown by arrows the paths of air-flow through the incubator for each of the three systems: A, the closed respiration system ———→; B, the open respiration system - - - - →; and C, the open system without respiration apparatus · · · · →. In the system A the absorbent (1: a, b, c, d and e) and the oxygen apparatus (2: a_1, b_1, c_1 and d_1) work simultaneously; in the system B the absorbent apparatus (1) works only; and in the system C the general condition is provided very close to that in commercial incubators, but more perfected. That is, outdoor fresh air, regardless of weather condition, is drawn by the blower (6) through the room-heating coil (5), the humidifier (11), the incubator-heating coil (17), and then, fully conditioned in respect to the temperature and humidity, through the egg chamber.

The egg chamber of the incubator, capacity 160 eggs, is air-tight, well insulated from the surrounding atmospheric conditions, and further, under the automatic control of the important physical factors of incubation: namely, heat, moisture, air, ventilation and movement of eggs. In use of the system C, for instance, these factors have been accomplished as follows.

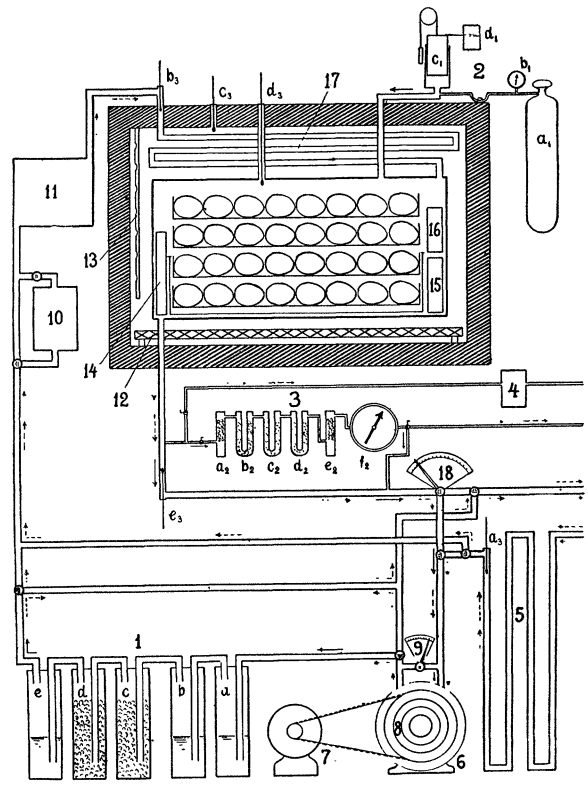

FIG. 1

The heat is supplied by the electric heater (12), regulated by the thermostat (13) and recorded by the thermograph (15) and the thermometers (a_3, b_3, c_3, d_3 and e_3). The moisture is supplied and regulated by the humidifier (11) and recorded by the hygrometer (16). The quality of air is regulated by means of changing from internal to external, or reverse ventilation at the valve (18), and determined in respect to the carbon dioxide content either by the residual system (3: a_2, b_2, c_2, d_2, e_2 and f_2) gravimetrically, or by the Haldane gas analysis apparatus (4) volumetrically, or by both. The ventilation is provided by the blower (6) driven by the electric motor (7), regulated by both the cone pulley (8) and by the by-pass valve (9), and recorded by the gas meter (10). The movement of eggs is controlled by the electric turning device (14).

With the above-described incubator it was possible to obtain a good, uniform control of the physical factors throughout the period of incubation; it is hoped that the attempt to study external factors of

[1] A. L. Romanoff, thesis (Ph.D.), Cornell University, June, 1928.

incubation may be of value for the future development of artificial incubation and commercial incubators.

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SPECIAL ARTICLES

MENDELISM AMONG BACTERIA?

DURING the summer of 1926 at the American Museum's station for the study of insects we isolated a strain of yellow pigmented bacterium that was pathogenic to the wood fly *Lucillia serricatta*. This organism was described in the American Museum Novitates No. 251[1] as *Bacillus lutzii* Brown. At the time the true generic affiliation was doubtful but it was suspected from the appearance in old cultures of spheric refractile bodies and from the fact that very young cultures—three to six hours old—reacted Gram positive that it belonged in the genus Bacillus. Since then a prolonged study has shown the refractile bodies not to be true spores and, as the brief period of Gram positiveness disappeared after the seventh or eighth transfer, we now believe the organism to be a member of the Bacteriacaea probably in Flavobacterium. The generic nomenclature in bacteriology and the scope of these genera are so ill-defined in many cases that it is difficult to assign some strains to any group. We at least do not feel qualified to originate any new generic names until the present ones are more fully understood.

Early in the work it became apparently difficult to keep the strains free of a yeast-like organism. Another difficulty appeared in the loss of color in one of the substrains. Both, while annoying at the time, have been found to be merely phases in the life history that, if we are correct in our inferences, may prove to be of more than passing interest.

As startling and as improbable as it may seem, we are of the opinion that we have been witnessing an exhibition of a case of Mendelism among asexual organisms. This statement may at outset seem contradictory to fundamentals, since Mendelism entails inheritance from two parents. However, a moment's reflection will reconcile it to some extent. It is a well-known and fundamental fact that great numbers of asexual organisms go through a process of conjugation during which portions of the nuclear material are exchanged. This is true in both the plant and animal kingdoms and now we propose that it is true in the intermediate bacterial kingdom.

Briefly, the life cycle of *F. lutzii* as exhibited under laboratory conditions and observed by us is as follows:

When the substrat becomes of such a nature as to interfere with the normal mode of existence as a rod-shaped organism, the rods undergo a series of morphological changes that terminate in a resistive stage. If the change in substrat is gradual, as might be caused by the natural accumulation of wastes in an old culture, many filamentous forms are developed and seem capable of existing long after the rods have died off. Upon transplanting these filaments the resultant culture is always one of pure rod forms. However, if the introduction to toxic material is sudden and if that toxic substance is of a cumulative type such as lead, a rapid change different in character occurs. A group of rods, eight in number we believe from lengthy observation, gather together and fuse into a mass. This mass is far more intensely stained by 1:10 aqueous basic fuchsin than the individual rods themselves are. This stage has been called by Löhnis[2] a symplasm.

While within this protective envelope the bacterial mass completely fuses and then gradually subdivides into two ill-defined parts and next into four spheric stain-resisting bodies. Each of these spheroids then passes through fission at right angles and tetrads are produced. These tetrads disintegrate into sixteen resistant "cocci." In time or upon being transplanted to a non-toxic nutrient medium each "coccus" again divides once, the increased volume due to this division causes the envelope to rupture and the "cocci" are released. If the substrat is suitable, they grow at once into normal rods and reassume the ability to take up aqueous basic fuchsin. These rods are of identical form, size, physiological and serological character with the initial rods.

If a single asexual "species" embraces strains varying in a character, such as color in our case, and if that character be Mendelian in its makeup, it is wholly conceivable that a stage of conjugation or, as the analogous bacterial stage may be called, symplasmism would bring this to light if carefully followed up. Apparently the difficulty heretofore obscuring its recognition has been the lack of a strikingly recognizable variable character. Our color character was ideal for this work as it was very definite and easily recognized.

Originally we set out to determine the cause of the loss of color in the substrain noted above. We at first believed it to be some obscure environmental cause and set out to eliminate as many as possible. We had found that by making daily transfers all strains kept their original color and that the only form present was rods. However, in the white strains

[1] F. M. Brown. American Museum Novitates No. 251. 1927.

[2] F. Löhnis and N. R. Smith. *Jour. Agric. Research*, 5: 676–702, 1916; *Jour. Agric. Research*, 23: 401–432, 1923.