

diluted milk and the fewest in that containing the most water.

IV. When suspended in aqueous mixtures containing from 5 per cent. to 42 per cent. of chemically pure glycerin and held at  $-20^{\circ}\text{C}$ ., a very large percentage of *B. coli* remain alive for at least six months.

V. At  $+37^{\circ}\text{C}$ ., *B. coli* in water or in 5 per cent. to 20 per cent. glycerin<sup>2</sup> die rapidly, few if any remaining alive at the end of 72 hours. The death rate diminishes as the holding temperature is lowered, though it is still marked even just above  $0^{\circ}\text{C}$ .; but at a temperature slightly lower a sudden change appears, the death rate at and below that point being but little, if any, greater than at  $-20^{\circ}\text{C}$ .

VI. By covering a 24-hour growth on agar with a sterile 10 per cent. cane sugar solution, and holding at  $-10^{\circ}\text{C}$ ., stock cultures of *B. subtilis*, *B. aurococcus*, *B. megaterium*, *B. fluorescens*, *B. proteus* and *Sarcina aurantia-cus* have been kept in a vigorous condition (without transferring) for eight months.

From these results the following conclusions may be drawn:

Low temperatures alone do not destroy bacteria. On the contrary, they appear to favor bacterial longevity doubtless by diminishing destructive metabolism. Frozen food materials, such as ice cream, milk and egg substance, favor the existence of bacteria at low temperatures, not because they are foods, but apparently because they furnish physical conditions somehow protective of the bacteria.

It seems likely that water-bearing food materials as well as sugar solutions, glycerin solutions, etc., freeze in such a way that most of the bacteria present are extruded from the water crystals with other non-aqueous matters (including air) and lie in or among these matters without being crushed or otherwise injured; while in more purely watery suspen-

<sup>2</sup> Glycerine mixtures much exceeding 20 per cent., at temperatures above the freezing point of water, act as mild antiseptics. Under 20 per cent. this is not the case, the death of the bacteria apparently resulting from lack of food, as it does not occur when a small amount of peptone is present.

sions, and, above all, in water itself in which the whole mass becomes solidly crystalline, they have no similar refuge but are perhaps caught and ultimately mechanically destroyed between the growing crystals. This theory would explain the absence of live bacteria in clear ice, their comparative abundance in "snow" ice and "bubbly" ice, and also the fact that the more watery food materials when frozen contain the fewest, and the least watery the most, living bacteria.

The comparatively rapid death of bacteria in non-nutrient materials at higher temperatures and their slower dying at lower temperatures agrees well with the theory of simple starvation or destructive metabolism. At the higher temperatures they perish quickly because they burn themselves out quickly; at the lower, more slowly, because they consume themselves more slowly. At temperatures where metabolism ceases altogether they continue to exist in a state of suspended vitality similar to that exhibited by many other and higher plants which in the far north are subjected without apparent injury for long periods to temperatures much below the freezing point of water.

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#### HEMOPHORIC FUNCTION OF THE THORACIC DUCT IN THE CHICK

IN a recent investigation of the development of the thoracic duct in the common fowl, the writer studied also certain aggregations of mesodermal cells correlated with the developing duct, and considered by Sala,<sup>1</sup> more than ten years ago, as "cords" of mesenchymal cells out of which were "hollowed" the rudiments of the duct.

The writer believes, and in the near future will publish evidence to substantiate the belief, that these aggregations of mesodermal (mesenchymal) cells comprise developing blood cells which are differentiated *in situ* out of the indifferent mesenchymal syncytium, that these blood cells then gain access to the lymph

<sup>1</sup> Sala, *Ricerca fatta nel Lab. di Anat. Norm. della R. Univ. di Roma*, Vol. 7, 1900.

channels making up the developing thoracic duct, and that finally the hemal cellular elements in question reach the blood stream *via* the thoracic duct and jugular lymph sac. Considering the vast number of blood cells, especially erythrocytes, arising in this region and the probability that they are conveyed to the general circulation by the thoracic duct, this duct assumes, therefore, an additional phase of importance in the chick in that it performs a hemophoric, or blood-carrying, function.

The view that the thoracic duct may arise as detached portions of veins is in the case of the chick quite untenable, since the tissue in which the lymph spaces and channels arise remains notably non-vascular up to the time the first lymphatics appear. The writer believes he has sufficient evidence, soon to be published, to show that the lymphatics arise as isolated lacunæ directly from mesenchymal intercellular spaces, are not in any sense derived from veins, and subsequently coalesce to form the continuous channels of the thoracic duct.

The point recently made by other investigators,<sup>2</sup> namely, that the superficial lymph plexus in the region of the posterior lymph heart in the chick contains stagnant blood which has backed up into it from the veins, is invalid in the case of the thoracic and its blood content because there are no veins in this region from which "backing up" could occur.

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#### A POSSIBLE MEANS OF IDENTIFYING THE SEX OF (+) AND (—) RACES IN THE MUCORS

It has been shown by the writer (1) that the majority of the forms among the mucors are dioecious, with the sexes separated in male and female races which are capable of being propagated apparently to an indefinite number of vegetative generations by means of nonsexual spores formed in sporangia. In all the dioecious species carefully investigated the opposite gametes, which are produced and unite to form zygosporangia when the two sexual

races of a given form are grown together, do not appear to differ morphologically. Lacking a definite criterion which an inequality of the gametes would have afforded, the writer has provisionally designated the opposite sexes in these forms by the signs (+) and (—) on account of a generally greater vegetative<sup>1</sup> luxuriance of one sex over the other. That in reality the two sexes are represented in the (+) and (—) groups is shown by the sexual reaction which may occur not only when the (+) and (—) races of the same species are grown together and perfect zygosporangia are produced, but also by the sexual reaction which may occur when (+) and (—) races belonging to different species are grown together. This reaction between the opposite races of different species has been called imperfect hybridization since it does not lead to the formation of perfect hybrid zygosporangia, but usually stops short with the formation of progametes, though occasionally gametes are produced which, however, never unite.

A sexual race of a dioecious species if grown between the (+) and (—) races of another test species used as a standard, will show a line of sexual reactions on one side only. Some of the hermaphroditic species, on the other hand, when similarly grown, show a response to both (+) and (—) test races and produce therefore 2 lines of sexual reactions.

Some few species in the hermaphroditic group are distinctly heterogamic with a constant difference in size between the conjugating gametes. Figs. 1-6 in the accompanying diagram will illustrate the process of conjugation in such forms. It seems reasonable to consider the larger gamete female and the smaller male. Upon this basis, if a sexual reaction could be established between these unequal gametes and the (+) and (—) races, the race reacting with the larger female gamete must be considered male, while the race reacting with the smaller male gamete must be considered female.

<sup>1</sup>"Zygosporangium Formation a Sexual Process," SCIENCE, N. S., 19: 864-866, 1904; "Sexual Reproduction in the Mucorineæ," *Proc. Am. Acad.*, 40: 205-319, pls. 1-4, 1904.

<sup>2</sup>Clark, E. L., *Anat. Record*, Vol. 6, No. 6, 1912; Clark, E. R., *Anat. Record*, Vol. 6, No. 6, 1912.