shell gains an electron, while in alpha decay, both nucleus and valence shell lose a pair of electrons each.[2]

INGO W. D. HACKH

COLLEGE OF PHYSICIANS AND SURGEONS,
SAN FRANCISCO, CALIFORNIA

THE SUGAR TOLERANCE OF YEASTS EXPRESSED IN ATMOSPHERES

MYCOLOGISTS who study microorganisms on culture media are presented with physiological problems, such as the osmotic relations of microorganisms to their substrata. The plant physiologist considers and observes such relations in single cells and in turn in groups of cells. The microbiologist studying yeasts has a good opportunity to appreciate the interplay of osmotic forces outside and inside cell membranes, because of the advantageous size of these organisms.

In considering the osmotic relations of any cell or group of cells we get a true picture of natural conditions, if we think of each cell, tissue or organism as part of a moist system. We think then of the substratum and the yeast cell, for example, as in a continuous relation through moisture or water. Such continuity of water relations is absolutely essential for all organic life. We are apt, however, to think of a fungous body as merely displacing a volume of the solution in which it occurs. If we regard this displacement as anything but the occurrence of an instant we do not have a true picture. We had best think first of the continuity of the moist environment of the microorganisms as being throughout their cell structure.

The environment in which we culture our microorganisms is generally of the density or water content presented by dextrose broth or agar, which is in turn theoretically influenced by the moisture content of the air in the test-tube. Microorganisms growing on agar slants absorb or give off solutes only and therefore are most intimately related to organic substances which became solutes in a moist environment. There is no exchange between the protoplasm of a cell and its environment except as substances in solution.

For a further realization of this principle, picture yeasts or molds in juxtaposition to grains or particles which are of such a nature as to be unchanged by any reaction. Picture these yeast cells and such inert particles as part of a moist system. The moist environment is continuous throughout the yeast cell. The inert particle is unaffected. There is no interplay of osmosis between the living cell and the inert grain. Replace the particle with a sugar crystal of the purest type. The moist environment is now continuous with the crystal as well as with the living cell. There can no longer be the inert relation which existed between the yeast cell and the inert particle. The living cell, the syrup films of the crystal and the crystal must adjust their osmotic relations, and this continually. Life is not an equilibrium, but is continually trying to approach it. It is always getting there. When we admit these conceptions, we must admit that all microorganisms which carry on metabolic and reproductive activities in concentrated solutions have a higher tolerance of high osmotic pressures than those subsisting only in dextrose broth of the standard methods or in the liquid phase of a solid culture medium.

What specific proofs are there of such a relation pertaining to yeasts tolerant of concentrated sugar solutions? The following instances are illustrative and sufficiently indicative. When we use water blanks instead of a sugar solution for dilution blanks in count work, with sugars the count is lower, because some cells are killed by the dilution water. We may also observe the same relationship when we take a loop of growing yeasts from a sugar solution of 50 per cent. total solids and add to it water or more dilute sugar solution to complete a microscopic mount. Again almost instantaneous plasmolysis occurs, when we mount living yeast cells growing on a sugar agar slant of 50 parts of sugar to 100 parts of agar medium in water or broth containing 35 parts of sugar per hundred. Further, yeasts grown at as high a concentration as 50 parts of sugar to 100 of broth or agar medium are smaller than when grown in 35 parts of sugar to 100 parts of broth or agar medium. Pfeffer[1] claims that as cells become acclimated to concentrated solutions the cell wall develops strength and the radius of the cell decreases as the cell's resistance to osmotic pressure increases. Cells thus accommodating themselves to concentrated nutrient solutions which constitute their environment must be inherently able to increase the amount of osmotic substance they contain. With such accommodation the appearance of the protoplasm changes. We have observed excessive vacuolation in yeasts growing in high sugar solutions.

The density of sugar solutions may be expressed as atmospheres. Considering the usual broth media, we find that from $1\frac{1}{2}$ to 3 per cent. of sugar is their usual concentration. A 1 per cent. dextrose solution would have its osmotic pressure expressed in atmospheres by a value of 1.25. Dextrose broth containing 1 per cent. of sugar would be somewhat higher, due to added nutrient, as beef extract or peptone. The inoculated medium, due to the inclusion of the sam-

[2] Hackh, *Phys. Rev.*, vol. 13, p. 165, 1919; and *Phil. Mag.*, vol. 39, p. 155, 1920.

[1] W. Pfeffer, trans. by A. J. Ewart, "Physiology of Plants," Vol. I, pp. 136, 140. 1906.

ple, would be somewhat higher in value expressed as atmospheres. Plant cells when turgescent have a cell sap represented by 5 to 10 atmospheres. Beet sugar sap has a value of 15 to 21 atmospheres, or that of a 25 to 30 per cent. solution of sucrose. Fungi as Penicillia and Aspergilli have been grown in concentrated solutions having a value of 157 to 160 atmospheres, or an equivalent of 45 per cent. KNO_3.[2] Owen[3] in Louisiana was able to grow yeasts in molasses having a density of 70 per cent. total solids or when figured as sucrose the equivalent of 225 to 260 atmospheres. As molasses contains lower sugars and gums the figure is actually higher. We have grown yeasts in culture tubes where the medium was 62 per cent. total solids, which may be represented by 140 to 180 atmospheres figured as sucrose. The actual figure is higher for the reason stated above. Also we have grown yeasts in hanging drops of mixed syrup having a density of 70 per cent. or again expressed in atmospheres 225 to 260. Yeasts did not grow in the purest cane syrup (c.p.) of 68 per cent. total solids. Cream centers of chocolate coated candies inoculated with sugar tolerant yeasts burst after a period of storage normal for ripening such confections. The syrup density of the expressed syrup phase of the cream of fondant was 76 to 77 per cent., or greater than 225 to 260 atmospheres.

Summarizing, we may state that yeasts apparently do not grow in solutions of mixed sugars having a density of 79 per cent. This concentration appears to indicate the cessation of activity for sugar tolerant yeasts. Investigators at the Michigan Agricultural Station have apparently reached the same conclusion.[4] We need to bear in mind, however, that between cessation of reproductive development and cessation of all activity there is a period where enzymatic activity of living cells and finally autolysis of dead cells progresses.

MARGARET B. CHURCH

URBANA UNIVERSITY,
URBANA, OHIO
August, 1931

MORE DATA ON THE LUNG FLUKE, PARAGONIMUS, IN NORTH AMERICA

WALLACE,[1] of the University of Minnesota, has recently found that the mink is the normal definitive host of *Paragonimus* in North America. He examined minks from fur farms. He included a small amount of life history data in his report and in a second article[2] gave a more detailed account of this phase of the work. The work here reported was begun in March, 1930, independently of and without knowledge of Wallace's work. My records show that metacercariae were discovered June 22, 1930, and mature worms secured on September 4, 1930, from an experimentally fed cat. The mink was determined to be the normal definitive host in North America without knowledge of Wallace's contribution which was published after my survey of the fur-bearing animals was completed.

In the fall of 1929, in connection with another problem, a cat was used that had come from Platt, a small village on the outskirts of Ann Arbor. Examination of its feces at the time revealed the characteristic eggs of *Paragonimus* and this diagnosis was verified several months later at the death of the cat when eight adult worms and a large number of eggs were removed from the lungs. On the basis of this discovery, a search for the metacercaria was begun the following spring when various species of crayfishes from local ponds, lakes and streams were examined and on June 22, 1930, a metacercaria closely resembling that of the Asiatic lung fluke was discovered in the pericardial region of 60 per cent. of the *Cambarus propinquus*[3] collected in Fleming Creek, a stream several miles east of Ann Arbor. Cysts were fed at intervals to a cat which developed the characteristic *Paragonimus* cough. On September 4, 1930, ten weeks after the first experimental feeding, the cat was killed and forty-three worms of various ages as well as thousands of eggs were recovered from the lungs. Subsequent examination of crayfishes from the same stream revealed that *C. robustus* also harbored the metacercariae but very few individuals were infected. The cysts are usually found in the wall of the heart or adhering to it, though occasionally some are found either attached to the tissues surrounding the pericardial cavity or free in the lumen of any of the large blood vessels leaving the heart. In heavy infections, the majority of the cysts occur in clusters arranged in a belt across the broad base of the heart. Even in the heaviest infections, the cysts are absent from the gills or muscles. In the fall, collections of stream crayfishes taken from all parts of the lower peninsula to determine the distribution of the worm in this part of the state, added another host species, *C. virilis*. Wallace found metacercariae in a single species, *C. immunis spinirostris*. Apparently any species of crayfish occurring in the appropriate environment will serve as an intermediate host though

[2] *Ibid.*, p. 140.

[3] Wm. L. Owen, "Swells in Canned Cane Syrup and Molasses," "Facts about Sugar," 21, pp. 946–9, Oct. 2, 1926.

[4] F. W. Fabian and R. I. Quinet, "A Study of the Cause of Honey Fermentation," Mich. Agr. Sta., *Tech. Bull.* 92, pp. 1–41, 20 figs, Feb., 1928.

[1] F. G. Wallace, "Lung Flukes of the Genus Paragonimus in American Mink," *Jour. Am. Vet. Med. Asso.*, 31: 225–234, 1931.

[2] F. G. Wallace, "The North American Lung Fluke," SCIENCE, n. s., 73: 481–482, 1931.

[3] The writer is indebted to Mr. E. P. Creaser, of the University Museums, for identification of crayfishes.