bottle and egg with a beaker just tall enough to clear the egg, the embryo would develop from the twenty-fourth to the hundred and tenth hour of incubation. The embryo came into view at the edge of the exposed surface very soon after the shell was removed. Mortality was rather high.

Up to this time, we had been unsuccessful in developing normal embryos in eggs from which such an area of shell had been removed prior to incubation. But at this point we found that by retarding evaporation still further by plugging the tumbler or beaker used as a cover loosely with a towel or with cotton it was a very simple matter to observe approximately normal development in the embryo from the unincubated stage to about one hundred five hours incubation. These chicks die at a remarkably uniform age; they do not die from the direct effects of evaporation. The causes of their death are being investigated in these laboratories at present.

This method makes it possible for any undergraduate student to study the first four days of the development of the chick embryo in the same chick, to catch any desired stage for histological study, and that without the mastery of a difficult technique or a supply of expensive apparatus. It is almost superfluous to point out the added ease of experimentation that this technique offers the investigator of the early developmental physiology of the chick embryo.

The materials required are: 1 tumbler, 6″ x 2.5″; 1 straight-side bottle, 3″ x 1.5″, and one small towel. The bottle and tumbler should be washed with 95 per cent. alcohol; further sterilization has so far been unnecessary. The towel should be crumpled and placed beneath the bottle containing the egg and all three inserted into the tumbler until the surface of the egg almost touches the bottom of the tumbler. The assembled apparatus is placed in the incubator and may be removed for observation at will. Frequent dissembling of the apparatus for brief intervals to expedite closer inspection seems to do no harm.

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SPECIAL ARTICLES

THE ORIGIN OF VACUOLES

DEVELOPMENT of vacuoles in the cytoplasm of plant and animal cells is not uncommon both under experimental and natural conditions. As regards plant cells our own historical approach to the subject may be cited to illustrate the fact.

For many years our students have been instructed to study a vacuolization of the cytoplasm that chromates and dichromates in plasmolyzing concentration produce, *e.g.,* in the epidermal cells of onion bulb scales.[1] Later, a similar frothing of the cytoplasm in various cells was noted to occur with any plasmolytic agent after preliminary action of trivalent cations even in very dilute solution.[2] In sufficiently high concentration, however, even the most innocuous plasmolytes by themselves may cause subsidiary vacuoles to arise in the cytoplasm—a matter of common observation.

It is not only with plasmolyzing agents that this effect is produced, but also with other more readily penetrating substances, *e.g.,* narcotics such as chloroform and ether and by salts after exposure to very low concentrations (1 per cent.) of these. The outer surface and also the interior of the chloroplasts are common situations for the vacuoles to arise when produced in this way, as was observed even by von Mohl.

But without any artificial influence similar vacuoles may form in normal cells. One of us recently demonstrated their constant occurrence in the gametes during the conjugation process in *Spirogyra*[3] and further proved their excretory function as exercised in the taking up of water from the central vacuole and its discharge to the exterior in typical "contractile" fashion. The same author has recently found *Vampyrella* to be comparable in a remarkable degree to the gametes of *Spirogyra,* in that rapid excretion of water takes place by the activity of numerous contractile vacuoles appearing anywhere in the hyaline zone of the body; and that in addition to water, solid excreta are ejected by the simultaneous action of small vacuoles dispersed beneath the entire free surface.

To this is now to be added two principal facts primarily observed by the other writer, but studied by both of us, *viz.,* (1) that the vacuoles produced under the action of a strong plasmolyzing agent are also contractile, and (2) that these vacuoles originate from peculiar bodies already present in the cytoplasm. These bodies, more fully described elsewhere, bear a strong resemblance to the growths of lecithin in water which have long been known as "myelin forms." They are normally of irregular and varying shape and consist of an external lipoid (osmic acid reducing) film which is usually liquid and extensible, enclosing apparently a more aqueous interior which is usually in circulation.

On treatment with a rather concentrated plasmolyzing agent, *e.g.,* 1M or .75M cane sugar, the irregular

[1] Lloyd, F. E., and Scarth, G. W., "An Introductory Course in General Physiology," Montreal, 1921.

[2] Scarth, G. W., "Adhesion of Protoplasm to the Cell Wall and the Agents which cause it," Proc. Roy. Soc. Can. Ser. II, *17*: 137, 1923.

[3] Lloyd, F. E., "Conjugation in Spirogyra," Trans. Roy. Can. Inst. *15*: 129, 1924.

bodies round up into small spheres which soon begin to swell and behave actively as contractile vacuoles. Since a fresh vacuole frequently starts up where one has disappeared it is possible that the evacuated membrane of one condenses to form the primordium of another. There is good reason to believe that sugar in the above concentrations enters the cytoplasm, where, by some process that we do not understand, it is probably concentrated in the contractile vacuoles, the resulting swelling and bursting being explicable simply as osmotic and surface tension phenomena. When, owing probably to high viscosity of the external surface of the protoplasm induced, *e.g.*, by chromates, etc., the vacuoles fail to burst, the cytoplasm becomes thickened into a foamy mass.

There are suggestions in the literature that an origin of vacuoles from similar bodies may be the rule in widely different cell types. For example, the production of secretory vacuoles from the so-called "Golgi apparatus" in cells of the Epididymis as described by Nassonov[4] and Ludford.[5] Now in *Spirogyra* one phase of the polymorphic myelin growths answers every description of the "Golgi apparatus." Mention may also be made of Bensley's account[6] based on a study of fixed materials of the evolution of the central vacuoles in onion roots from a canaliculate system which might well be identical with what we have described in the living cell.

To summarize, in the origin of vacuoles a portion of the living protoplasm which is enclosed in a film of lipoid substance enlarges in volume by the intake of water. At what stage the diluted protoplasmic substance ceases to be alive or whether the central vacuole may be part of the living system thus becomes a question analogous to that of the cell wall. There are grounds, however, for regarding the limiting film as not altogether dependent on the life of the cell for some of its most characteristic behaviors. As regards its growth the resemblances to the physical growths of lecithin is remarkable, and as regards semipermeability the lining of the sap cavity, which gives a similar lipoid reaction, may retain this property long after the cell is dead. This has been known since De Vries's "Plasmolytischen Studien," but we have recently observed extreme examples of the fact. In cells "killed" by iodine with eosin the vacuolar membrane contracted in concentrated glycerine; thereafter for 8 days it underwent slow deplasmolysis retaining its smooth contour, and, for a part of the time, maintaining a high concentration of eosin, indeed much higher than on the outside. Recently we have noted that the tonoplast can retain its smooth contour also after sufficient treatment with osmic acid vapor to flocculate the contents (in part) of the central vacuole.

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[4] Nassonov, D., Archiv. fur mik. Anatomie, *100*: 1924.

[5] Ludford, R. J., Proc. Roy. Soc. B. *98*: 354, 1925.

[6] Bensley, R. R., through Cowdry's "General Cytology," p. 343.

THE TRANSFER OF EXCITED ENERGY FROM OZONE TO HYDROGEN AND NITROGEN

FRANCK and Cario[1] have shown that energy may be transferred from photosensitized mercury atoms to hydrogen at low pressures. The behavior of the excited hydrogen leads these workers to conclude that the active gas is hydrogen atoms. Bonhoffer[2] has made a study of the decomposition of ozone by photosensitized chlorine and bromine to determine a relation between the absorbed radiant energy. Rideal and Norrish[3] have used the photosensitization of ozone decomposition by chlorine in a determination of the kinetics of the reaction between hydrogen and oxygen. Taylor and Marshall[4] give the results of their work on the reaction of hydrogen atoms, produced by excited mercury atoms, with a variety of gases, including nitrogen. The hydrogen and nitrogen used was freed from oxygen. Mixtures of nitrogen with excess hydrogen when illuminated with resonance radiation in the presence of mercury vapor showed little or no change in pressure and the tests with Nessler's reagent at the close of the runs were negative. However, Noyes[5] reports that ammonia is formed in mixtures of hydrogen and nitrogen by transference of excited energy from mercury atoms to the molecules of the above gases. In the report by Noyes we are not informed if special precautions were taken to remove oxygen except in his investigation using mixtures of hydrogen and nitrogen in contact with vapor of boiling mercury. In this case no ammonia was formed when oxygen had been removed from the gas mixture previous to its contact with mercury vapor. Dickinson[6] by using the method of Franck and Cario for making atomic hydrogen has shown that excited hydrogen atoms combine with oxygen at 45° C. This work has been extended by Mitchell,[7] who finds that the rate of the reaction between illuminated hydrogen and oxygen in presence of mercury vapor depends upon the pressure of the oxygen; and also that this reaction is retarded in the presence of argon. In the conclusion, he suggests the possibility that the active hydrogen is not atomic. These investigations described above have been conducted largely at low pres-

[1] *Zeit. Physik.*, *12*, 162 (1922).

[2] *Zeit. Physik.*, *13*, 94 (1923).

[3] *Jr. Chem. Soc.*, *127*, 787 (1925).

[4] *Jr. Physical Chem.*, *29*, 1140 (1925).

[5] *Jr. Am. Chem. Soc.*, *47*, 1003 (1925).

[6] Proc. Nat. Acad. Sci., *10*, 409 (1924).

[7] Proc. Nat. Acad. Sci., *11*, 458 (1925).