this reason were given. It will be interesting to note the manner in which Mr. Nash in the future parts of the work solves the various difficulties which will beset him in fixing the types of the genera.

The name *Tripsacum acutiflorum* Fourn. (*Bull. Soc. Bot. Belg.* 15: 466. 1876) is accepted in place of *T. lanceolatum* Rupr. (Fourn. Mex. Pl. 2: 68. 1881). This is based upon the statement made by Fournier, in a discussion of grasses with separated sexes, that in *Tripsacum* the peduncle of the male spikelet, ordinarily free, "est soudé avec le rachis de l'épi dans le *T. acutiflorum* n. sp." This statement certainly does not distinguish *T. lanceolatum* from the other species and can scarcely, therefore, be considered as sufficient to constitute publication. It is rather to be taken as incidental mention within the meaning of the American Code of Botanical Nomenclature (Canon 12. A name is not published by its citation in synonymy, or by incidental mention). The allies of *Rottbœllia cylindrica* have difficulty in keeping their names. When Otto Kuntze showed that the type species of *Manisuris* was a *Rottbœllia,* the names of these species were changed from *Rottbœllia* to *Manisuris.* Now Mr. Nash decides that this group is not congeneric with the type of *Manisuris,* but belongs to the genus *Stegosia* and the species are all transferred to the new allegiance. At the same time that Kuntze made the disconcerting discovery mentioned above he found it necessary to change the name of the grass generally called *Manisuris granularis,* since it obviously was not a true *Manisuris.* He called it *Hackelochloa granularis,* and is followed in this by Mr. Nash (and also by the present writer. See "Grasses of Cuba"). However, it appears necessary to take up for this genus the name *Rytilix* Raf. (*Bull. Bot. Seringe,* 1: 219. 1830).

While Mr. Nash's contribution is not, and could not be expected to be, monographic, it will be, when completed, of great service to agrostologists.

A. S. Hitchcock

SPECIAL ARTICLES

RHYTHMICAL ACTIVITY OF ISOLATED HEART MUSCLE CELLS IN VITRO

In previous communications[1,2] I pointed out that the heart muscle of chick embryos will beat rhythmically for many days when suspended in the media of a tissue culture and from such transplanted tissue there is an active growth of cells into the surrounding media. Braus[3] has repeated these experiments, using the hearts of embryo frogs and toads and he has found that these isolated beating hearts react to electrical and chemical stimuli similar to the intact heart. Braus also noted that the cells which grew from the hearts of cold-blooded animals were living at the end of three months. Very recently, Carrel[4] by the use of the method of repeated transplantation of the tissue from a culture to a fresh medium (Carrel and Burrows) has attempted to prolong the life and function of heart muscle *in vitro.* His experiments show that the rhythm which I noted in fragments of embryonic chick hearts can be prolonged, although intermittently, for a period of 85 days. The results of these experiments substantiate, therefore, the former well-known fact, namely, that strips of heart muscle, both of cold and warm blooded animals (Erlanger), will beat for some time when placed in the proper media. In none of these cases could one rule out, however, the possibility of the existence of nerve ganglia or some possible precursor in the young embryonic hearts, which might initiate rhythmical contractions.

During the present year experiments have been made to determine the conditions which would prolong the life and allow the development of functional activity in the cells which had grown and differentiated in the culture.

[1] Burrows, M. T., 1911, *Jour. Exp. Zool.,* Vol. 10, 63.

[2] Burrows, M. T., 1912, *Anat. Record,* Vol. 6, 141.

[3] Braus, H., 1912, *Weiner Med. Wochschr.,* No. 44.

[4] Carrel, A., 1912, *Jour. Exp. Med.,* Vol. XV., 516.

These experiments have shown that the newly grown, cellular syncytia and the isolated single heart muscle cell can become functionally active, beating with a rhythm similar to that of the intact heart.

Pieces of the hearts of chick-embryos of all ages and of young hatched chickens were used. A growth of tissue, composed almost entirely of muscle cells, occurred from all pieces when suspended in the media of both types of cultures, (1) the ordinary hanging drop culture (the plasma modification[1]) of the method of Harrison[5] and (2) a large modified type of culture. This apparatus is so arranged as to supply the tissues continuously with fresh media and to wash away the waste products without in any way disturbing the growing cells. I described this method in detail before the American Association of Anatomists, December 27, 1911.[2] Serum was used as the fluid medium in the latter type of culture.

Rhythmical activity of the newly grown cells was noted in 3 out of 15 of the large type of cultures (No. 2), and in 2 out of 150 of the ordinary hanging drop cultures. These cells were located definitely within the clot and had a clear cytoplasm which contained very few fat droplets. The rhythmical activity did not occur during the active outwandering of the cells but, later, after they became permanently located in a definite portion of the clot and were undergoing slow multiplication and differentiation. In one culture rhythm occurred as early as the fifth day, while in others as late as the fourteenth day of the life of the culture. The greater number of positive results in the large type of culture (No. 2) can be associated with the active and continuous growth of the tissue over a sufficient period of time. Active growth and a regular rhythm has been observed in these cultures for 30 days, while in the hanging drop culture the active growth and the regular rhythm cease after the third or fourth day. The growth then becomes gradually less and the rhythm intermittent, ceasing entirely after 10 or 18 days unless the tissue is transferred to a new medium. The method of repeated transplantation from the culture to a new medium has not as yet been sufficiently developed to allow any increase in the life and the activity of the newly grown cells. At each transfer of the tissue the actively growing and multiplying cells are destroyed and a new growth takes place from those more latently active cells in or about the tissue mass.

The original pieces of heart muscle transplanted to a tissue culture vary as to their rhythmical activity in relation to the portion of the heart from which they are taken as well as the age of the embryo. Pieces of the auricle, especially of that part situated near the entrance of the veins, taken from embryos of all ages and from young hatched chickens, beat when suspended in plasma. The pieces of the ventricle do not beat when taken from embryos older than 10 days, unless special methods of preparation and treatment are used.

Rhythmically beating cells have been grown from the contracting pieces of the hearts of young embryos and from one piece of the ventricle of a fourteen-day chick embryo. The absence of movement in the original mass of tissue of this culture facilitated greatly the study of the delicate contractions of the newly grown cells. The syncytial network which surrounded the original tissue and one isolated cell were beating rhythmically. This cell was situated far out in the clear medium away from all other tissues and beat with a rhythm independent in phase from that of the syncytium. The rate of all beating cells in this culture was the same, 50 to 120 per minute, or a rhythm typical for rhythmical beating pieces of ventricular muscle.

The experiments show: (1) that the cells which have grown and differentiated in a tissue culture can later assume their characteristic function; (2) that rhythmical contraction similar to that observed in the embryonic heart can occur in an isolated and single heart muscle cell; (3) that the rhythmically con-

[5] Harrison, R. G., 1907, *Proc. Soc. Exp. Biol. and Med.*, 140; 1910, *Jour. Exp. Zool.*, Vol. 9, 787.

tracting cells can be grown not only from the pieces of hearts of young embryos, but from the heart muscle of a fourteen-day chick embryo.

These experiments, therefore, give direct evidence for the myogenic theory of the heart beat.

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On Molecular Cohesion. A Preliminary Statement

There is much uncertainty both about the laws and nature of molecular cohesion. The attraction has been supposed to vary inversely with the square, the fourth, fifth, seventh, or even the ninth power of the distance between molecular centers; and whether cohesion is of the nature of magnetic, electric, or gravitational attraction, or whether it is of a kind of its own, is uncertain. Its relation to gravitation, on the one side, and to atomic affinity, on the other, is unknown.

1. *The Derivation of the Value a/V^2 in Van der Waals's Equation.*—The value of a/V^2 in Van der Waals's equation represents molecular cohesion. If each molecule has a mass of cohesion, M, and if the molecules attract each other inversely as the fourth power of the distance, as Sutherland suggests, then the attraction between two molecules is $M^2K/v^{4/3}$, v being the volume of one molecule. If there are $1/v^{2/3}$ molecules in a surface of one sq. cm. of a gas or liquid, the pressure per sq. cm. will be M^2K/v^2. If each molecule attracts only its neighbors, owing to the fact that the cohesion does not penetrate matter, then the internal pressure will be the same as the attraction of each double layer of molecules and instead of M^2K/v^2 we may multiply numerator and denominator by N^2, where N is the number of molecules in the volume V. This makes N^2M^2K/V^2, which is the value a/V^2 of Van der Waals's equation. It has the advantage over the usual form, a/V^2, in that the various constituents of "a" appear at once.

2. *The Latent Heat of Vaporization.*—Mills discovered the empirical relationship that the internal latent heat of vaporization divided by the difference of the cube roots of the densities of the liquid and vapor was a constant, except near the critical temperature. His equation was: $L - E_e = K(d^{1/3} - D^{1/3})$. He assumed that the internal latent heat of vaporization, or $L - E_e$, where L is the total latent heat and E_e that part of it consumed in doing external work, represented only the energy consumed in separating the molecules. He was struck by the resemblance of this equation, when transformed into $L - E_e = K'(1/v^{1/3} - 1/V^{1/3})$, to that of Helmholtz representing the heat given out from the sun on contraction from the radius CR to the radius R, or $3M^2K(1/R - 1/CR)/5$. The latter equation is derived by the gravitational law. Mills, therefore, concluded that the attraction of molecules must also follow the gravitational law and vary inversely as the square of the distance. The error in Mills's reasoning is the assumption that $L - E_e$ represents only the work of overcoming molecular cohesion. It represents not only this but also the heat consumed by the expansion of the molecules from their volume in the liquid to their volume in the vapor, for the molecules certainly expand on passing from the liquid to the vapor. If the heat thus consumed by molecular expansion is E_m, then since the difference in molecular cohesive energy in the vapor and liquid is $N^2M^2K(1/v - 1/V)$, $L - E_e = N^2M^2K(1/v - 1/V) - E_m$. Near the critical temperature E_m becomes nearly zero, and at the critical temperature this goes into the form $L - E_c = N^2M^2K(1/v - 1/V)$. Since the heat rendered latent by the expansion of the molecules increases as we go downward from the critical temperature, the value $L - E_e$ must become constantly greater than $N^2M^2K(1/v - 1/V)$, by the amount E_m. This is found to be the case. For example in methyl propionate $(L - E_e)/(d - D)$ has the following values in absolute units taking gram mol quantities:

Temperature	$(L-E)/(d-D)$	N^2M^2K/Wt
100°	3.417×10^{11}	
200°	3.030	