other two provinces. The normal marine junction of the Cataract and Brassfield seas is prevented by the Medina delta of sands. For these reasons, Medina, Cataract, and Brassfield are to be retained as names for independent marine faunas and formations. The details leading to these conclusions are set forth in a contribution entitled 'Medina and Cataract formations of the Siluric of New York and Ontario,' *Bulletin of the Geological Society of America.*

A METHOD OF OBTAINING COMPLETE GERMINATION OF SEEDS IN *OENOTHERA* AND OF RECORDING THE RESIDUE OF STERILE SEED-LIKE STRUCTURES

By Bradley Moore Davis

DEPARTMENT OF BOTANY, UNIVERSITY OF PENNSYLVANIA

Presented to the Academy, May 4, 1915

All genetical workers with *Oenothera* shortly become aware that generally only a small proportion of the seed-like structures sown in their seed pans produce seedlings before the pans are emptied to give place in the hot house for the developing culture. My own practice has been to keep seed pans from eight to ten weeks only, as it is uncertain, if sowings are made in January, whether seedlings appearing later can be brought to maturity during the summer. For the past three seasons I have counted the seeds sown and thus have obtained some information on the relative degrees of fertility in my cultures which in some cases have been surprisingly low. But this procedure does not give accurate data on the proportion of fertile seeds to sterile seed-like structures for the reason that germination of *Oenothera* seeds in earth is very irregular and may be delayed far beyond the time that it is convenient or possible to keep the seed pans. Seeds sown in earth are obviously lost for further enquiry as to the fact of their viability, a proportion of seedlings appears but, as for the residue, that cannot be examined.

The technical problem of obtaining from sowings of seeds cultures that will accurately represent the genetical possibilities of the sowing is under these conditions difficult. In *Oenothera* work this problem is vital for exact studies since through the delayed germination may be lost not only peculiar individuals but possibly, in the case of hybrids, entire classes of segregates. It is probably safe to say that no culture of *Oenothera* has as yet been described in which we may feel confident that all of the viable seeds have germinated. Consequently we cannot be certain that any of the reported percentages of 'mutants' or ratios of segregates from hybrids are correct. Furthermore it is important

to determine for oenotheras the ratio of fertile seeds to the structures that in outward appearance cannot be distinguished from seeds but which contain no living tissue or only a small amount of endosperm, with an embryo, if present at all, so small or abnormal that it cannot produce a seedling. We are indebted to O. Renner[1] for the first detailed investigation of the sterile seeds of an *Oenothera* and for the formulation of a most interesting hypothesis as the result of his studies on *O. Lamarckiana*.

During the past winter I have tested the seed fertility of fifty species, races, or hybrids of *Oenothera* and as a result of these experiments I have become convinced that genetical research in this group must in the future adopt methods that will ensure a rapid and complete germination of the viable seeds and at the same time conserve the sterile seeds or undeveloped seed-like structures for examination. Such methods will require that seeds be germinated in some convenient receptacle and subsequently be set in earth leaving as a residue within the receptacle all sterile structures.

The method employed this winter is briefly as follows: In a Petri dish $3\frac{3}{4}$ inches in diameter was placed a pad of circular filter papers 3 inches across and about $\frac{1}{4}$ inch thick. The dish, cover, and paper pad were then sterilized by heat after which boiled tap-water was added in such quantity that the thoroughly soaked paper pad lay in the center of the dish surrounded by water. The seed-like structures were then spread over the surface of the pad and the dish covered. The seeds, therefore, lay on a very wet surface and in a moist chamber; more water was added when evaporation materially lowered the level in the dish. The tap-water was boiled to avoid the introduction of algae; fungi appeared in some of the cultures but apparently found the seed coats an unfavorable substratum for they grew very little; bacteria were likewise not troublesome.

The Petri dishes with seeds were placed in shaded parts of the same hot house with cultures sown in earth and were thus under similar conditions as to temperature. The possible germination within the dishes was always very much more prompt than for a similar sowing of seeds in the earth and proved to be complete when sufficient time was allowed. The advantages of Petri dishes as receptacles are those of convenience in the removal of germinated seeds and in the collection and examination of the residue. Petri dishes are also readily stacked and upon labels over the covers may be recorded the data of the experiment.

The rate of germination in the Petri dishes under the conditions of

the hot house was irregular. Complete germination in some species required only three weeks, in other forms as much as from six to ten weeks, and in some cultures I found a few ungerminated seeds after three and one half months. In warm, sunny weather the house during part of the day was at 90°F., and these high temperatures were frequently followed by a burst of germinations. It is thus clear that the best results in experimental germination will be obtained when cultures are placed in chambers or incubators the temperature of which can be regulated with accuracy.

Hugo De Vries[2] has recently published a method of stimulating seed germination in *Oenothera* that seems likely to eliminate largely the irregularity and slowness of germination described above. He reports that well soaked seeds after being subjected to a pressure of 6–8 atmospheres for 1–3 days at room temperature will germinate in large percentages when removed to an oven at 30°C. His hypothesis for this interesting behavior assumes that the hard inner coats of the *Oenothera* seeds have narrow microscopic slits filled with air and that ordinarily water enters the seed with difficulty but under pressure can be forced to points where it may readily be absorbed. De Vries thus contributes the practical suggestion of an automobile pump and a strong chamber such as an autoclave into which receptacles containing the well soaked seeds may be packed. If complete germination is not obtained after one treatment to compressed air successive treatments alternating with normal atmospheric pressure and perhaps variation in temperature conditions may bring forward stubborn cultures.

The residue that is left after germination is believed to be complete has in my experience generally ranged from structures fully as large as the larger of the viable seeds to structures smaller than seeds, which in turn grade into the remains of numerous unfertilized, or abortive ovules, represented in the seed capsules by a light brown powder. It is an easy matter to determine whether or not the structures in the residue, seed-like in size and appearance, contain an embryo. The structures may be opened with the point of a needle or scalpel, or they may be squeezed flat between the tips of a strong pair of forceps. The presence of an embryo is at once made evident and the germ may be readily examined. Generally the seed-like structures will be found to be entirely empty or to contain only a trace of soft tissue. Structures smaller in size than normal seeds can be examined microscopically. The residue may be conveniently preserved as a record by arranging the structures on paper and covering them with a solution of shellac in alcohol, or with a solution of glue.

To illustrate the advantages of germinating *Oenothera* seeds under experimental conditions over the old practice of sowing upon earth I will give the results for a problem as attacked under the old and new method. In 1914 I germinated on earth seeds from an F_1 hybrid plant, 13.35ac, of the cross *O. franciscana* × *O. biennis*. A sowing of 819 seed-like structures produced a culture of 402 seedlings, a germination of about 50%. The culture was grown partly to test the inheritance of a character (red coloration of the papillae on the stems and ovaries) present in *franciscana* and absent in *biennis*. This character was fully dominant in the F_1 of this cross and in its reciprocal. It seemed reasonable to expect that a proportion of the plants in the F_2 generation would present the clear green stems and ovaries (recessive) of *biennis*, but I found no plants of this type in the culture of 1914. This winter (1915) I germinated in Petri dishes seeds from the same F_1 hybrid plant, 13.35ac, and obtained 761 seedlings from about 921 seed-like structures,—a germination close to 82%. The new method had raised the proportion of germination more than 30%. Of the 761 seedlings I was able to bring 748 to rosettes, the 13 which died probably belonging to a small group of etiolated dwarfs difficult to grow. It becomes a matter of interest to see whether or not in this culture of 1915 a group of green-stemmed plants will appear.

[1] Renner, O., Befruchtung und Embryobildung bei *Oenothera Lamarckiana* und einigen verwandten Arten. *Flora, Jena*, 107, 115 (1914).

[2] De Vries Hugo, The Coefficient of Mutation in *Oenothera biennis* L. *Bot. Gaz., Chicago*, 59, 169 (1915).

THE OSMOTIC PRESSURE OF THE IONS AND OF THE UNDISSOCIATED MOLECULES OF SALTS IN AQUEOUS SOLUTION

By Stuart J. Bates

CHEMICAL LABORATORY, THROOP COLLEGE OF TECHNOLOGY, PASADENA

Presented to the Academy, April 27, 1915

The concentration of the ions (C_i) and that of the undissociated molecules (C_u) are involved in the equation, $C_i^2 / C_u = K$, expressing the application of the law of mass-action to solutions of electrolytes. Hence the large divergence from this law which strong electrolytes exhibit may be due to the behavior of the ions, to that of the undissociated molecules, or to that of both. This so called anomalous behavior may be simply expressed by saying that for one or for both of these molecular species van't Hoff's law, $\Pi = CRT$, where Π is the osmotic pressure, C is the concentration in mols per unit-volume, R is the gas-