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## SPECIAL ARTICLES

## CENOTHERA NANELLA, HEALTHY AND DISEASED

In my cultures of the evening primrose of Lamarck, the pure and self-fertilized seed yearly produces a certain percentage of mutants, among them dwarfs, *Enothera nanella*, the number of which usually amounts to about 1 per cent. of the whole crop. Of late, these dwarfs have been the subject of some discussion, since Zeylstra discovered the presence of a bacterium in their tissues and showed that some of their characters, formerly considered as specific marks, are, in reality, abnormalities caused by this parasite.<sup>1</sup> From this, some authors have erroneously concluded that the dwarfs are no real mutants, but only diseased individuals of the original type.<sup>2</sup>

Zeylstra, however, had pointed out that, under favorable conditions, the sideshoots of the dwarfs may become healthy and lose their abnormal characters; but their height remains the same as in the diseased stems. Hence we may assume that, under still more favorable conditions, the main stems themselves might grow up healthy, while still retaining the dwarfish stature.

About half a century ago, Liebig pointed out that nitrogenous manure is apt to increase the sensitiveness of plants to diseases, whilst phosphate of calcium is one of the best means to diminish this predisposition. Laurent found the same to be true for such diseases as are caused by those common bacteria of the soil, which, under normal conditions, are harmless, but may injure the cultures, whenever the manure is too rich in nitrogenous substances. He studied *Bacillus fluorescens putidus* and *B. coli communis*, both of which destroy the cellwalls by means of their enzymes.

<sup>1</sup>H. H. Zeylstra, "Fzn. *Œnothera nanella* de Vries, eine krankhafte Pflanzenart," *Biolog. Centralblatt*, 1911, Bd. XXXI., pp. 129–139.

<sup>2</sup> Sammelreferat by G. Tischler, Zeitschr. f. ind. Abst., 1911, Bd. V., p. 327. even before they themselves reach the cells. The bacterium of *Enothera nanella* is of a wholly different type, since it is found within the living cells and changes their growth without killing them. Zeylstra provisionally placed it in the group of *Micrococcus*.

From these data it is probable that healthy O. nanella might be obtained by giving them less nitrogen and more phosphate of calcium. Unfortunately, however, the nitrogen manure acts as the strongest stimulant, under our climate, to induce them to become annual, and for many reasons it is most desirable to have cultures of annual generations. It is, therefore, necessary to determine the amount of nitrogen and phosphate of calcium which will induce a sufficiently large percentage to become annual, but will not essentially heighten their liability to become diseased.

In the summer of 1911, I made some provisional experiments which show that, by this method, there may be produced almost wholly healthy specimens with the normal stature of the dwarfs. In the first place, I found that every part of the stem, every single leaf and flower, may be normal or diseased, in response to external influences. In the young rosettes of rootleaves the first leaves were formerly always twisted; then came long-stalked normal ones and, after these, the really abnormal leaves with broadened and shortened bases. which often killed the terminal bud before it could make a stem. By giving a large amount of phosphate of calcium, and as little nitrogen as possible, every one of the rootleaves could be grown healthy, with a stalk and a narrow wedge-shaped base. The same was the case with the leaves of the stem, and even with the flowers. The number of the abnormal ones could be brought down to a very few, thereby giving the whole plant the appearance of a healthy condition. All transitions between diseased and normal dwarfs were to be seen in these cultures.

Moreover, I have won beautiful healthy dwarfs by means of a cross from which the other parent was eliminated after the rule of the sesquireciprocal crosses.<sup>3</sup> I pollinated **a** <sup>3</sup>('Ueber doppeltreciproke Bastarde,'' Biol. Centralbl., 1911, T. 31, pp. 97-104. dwarf of O. nanella  $\times$  biennis with the pollen of an ordinary O. nanella and got a culture of O. (nanella  $\times$  biennis)  $\times$  nanella == O. nanella which contained a high percentage of healthy plants. They began flowering when only 20 cm. high, the first flower appearing at a height of 10 cm.; whilst O. Lamarckiana reached 1.50 m. before flowering, the first flower opening about 80 cm. above the soil. All their leaves were as narrowly elliptical and as clearly stalked as those of the Lamarckiana itself, whilst the flowers were free from those abnormalities which usually accompany the dwarfish stature.

Thus we see that the discovery of Zeylstra, far from diminishing the value of *Enothera* nanella as a real and (in an experimental way) most useful mutant, has given the means of cultivating it in as healthy a condition as may be required. HUGO DE VRIES

## BEHAVIOR OF SPERMATOZOA IN PLASMA

THE recent article of Loeb and Bancroft<sup>1</sup> and of De Meyer<sup>2</sup> in which their observations upon the behavior of spermatozoa in various sorts of solutions, such as extracts of eggs of the same species (De Meyer, eggs of *Echinus* microtuberculatus; Loeb and Bancroft, eggs of the common fowl), colloids, acids, alkalies, hypo- and hypertonic solutions, egg-albumen, blood serum and Ringer solutions are described open up a most interesting field for investigation. During the past summer while occupying a table at the Marine Biological Laboratory, Woods Hole (for the use of which I am indebted to Professor F. R. Lillie), I attempted to grow spermatozoa of Arbacia punctulata, Mytilus edulis and Modiolus modiolus in various solutions, some of which being listed above as used by these other workers.

## <sup>1</sup> Journ. Exp. Zoology, 12: 381.

<sup>2</sup> Arch. Biol., 1911, Bd. 26, H. 1, pp. 65-97: <sup>('Observations et expériences relatives a l'action exercée par des extraits d'œufs et d'autres substances sur les spermatozoïdes.'' I have seen only Robert Lewin's review in the Zentralb. für Biochemie und Biophysik, XII., No. 19/20, of De Meyer's paper.</sup>

On August 2, I centrifuged Limulus blood plasm and made a hanging drop from the upper layer, which examination showed to be free from cells; into this drop I introduced a few sperms from Arbacia. Great difficulty was experienced in attacks of bacteria and many of the preparations were discontinued the following morning. The slides were sealed with vaseline, as in the usual culture mount, and left at room temperature. By the eighth of August there was no movement in the sperms, although it had persisted up until that time and therefore the copper component of the blood of this animal does not seem to be toxic for Arbacia sperms, but none of the phenomena about to be described from mounts in different media were observed.

On August 5, a culture was made in the sterile agar medium, made according to the customary bacteriological formula, diluted so that it was liquid but highly viscid at 20° C. The spermatozoa lived only a short time and were seen to disintegrate within 24 hours. It may be stated that the reaction of the agar was estimated only roughly by an indicator and not titrated, so that I am not certain whether the medium was suitable from this standpoint. Care was taken to render the seaurchins as free from bacteria as possible, the tests being washed off with HgCl., 1:1,000 before the cuts were made and sterile sea-water was used to receive the testes after extirpation. The mounts remained sterile throughout the time of observation, showing that the testes are bacteria-free, as one would suspect.

The plasma of a Norway rat was then tried on August 8 and this was prepared by centrifuging the blood of the rat in paraffin-lined tubes at about  $8^{\circ}-10^{\circ}$  C. The plasma clotted when the hanging drop was made at room temperature, but sufficient time elapsed before the plasma clotted for the introduction of the sperm. The behavior of the sperm-heads was discovered to be quite like that described by Loeb and Bancroft for the sperm of the fowl, for the heads enlarged, became less dense, and distinct chromatin granules were visible, even in unstained preparations, resembling the nuclei of the spermatids of certain insects