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The Site of Penetration Resistance to Water in Plant Protoplasts

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With 11 Figures

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Summary

The main purpose of this investigation was to determine the primary site of resistance to the penetration of water in the protoplasm of inner epidermal cells of the *Allium cepa* bulb scale. Since it is known that the tonoplast has a very high water permeability, it was left to decide whether the mesoplasm and/or the plasmalemma is the main barrier. According to a theory of HÖFLER, the mesoplasm is the main barrier. Because it is not possible to isolate the plasmalemma, the influence of the mesoplasm was removed by causing rosette systrophy. In rosette systrophy, almost all of the mesoplasm is collected arround the nucleus and the tonoplast and plasmalemma lie adjacent in the greater part of the protoplast.

Cells with and without systrophy are found in the same preparation but show no great difference in water permeability. The systrophied cells have even a lower water permeability constant than the non-systrophied cells. This indicates clearly that the mesoplasm is of no significant importance for water permeability, and that the primary site of penetration resistance to water is the plasmalemma.

It was possible to measure the water permeability constants of tonoplasts. While the $2\,K_{wo}$ values for protoplasts are approximately $6\text{–}8 \times 10^{-4}$ cm/sec, those for tonoplasts are about 100 times higher.

The water permeability constants found with glucose solutions were essentially the same as those found in solutions of $KCl + CaCl_2$. Other less inert substances, such as EDTA, give different (higher) values.

Using the method of partial deplasmolysis and plasmolysis, it was possible to change the protoplast volume several times, once until the eight time in K-Ca solutions and until the fifth time in glucose solutions.

The water permeability constants do not change appreciably, neither in the sequential plasmolysis steps nor between deplasmolysis and plasmolysis. Yet there is a small but significant difference between deplasmolysis and plasmolysis values. The deplasmolysis values are slightly higher.

In the K-Ca solutions the tonoplasts which were formed showed a linear expansion which indicates ion permeability. Permeability constants are $0.003\text{–}0.006 \times 10^{-4}$ cm/sec, about in the same range as those of moderate anelectrolyte permeability.

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1. Introduction

One of the oldest problems of cell physiology is the question of the site of penetration resistance in the protoplast (COLLANDER 1956).

Since PFEFFER's pioneer work, "Osmotische Untersuchungen", in 1877, it has been asserted that the outer plasma membrane forms the main barrier for permeation. PFEFFER says that a peripheral layer, the plasma membrane, decides about the uptake or non-uptake of a dissolved substance in the protoplasm (*l.c.*, p. 235).

The first achievement in the investigation of the plasma membrane (which has remained important until today) was the work of ERNST OVERTON (1895 to 1899). His anelectrolyte permeability investigations lead to the first theory of the molecular structure of biomembranes. He says (1899, p. 110): "Nach vielem Nachdenken neige ich immer mehr zu der Vermutung, daß das Cholesterin oder eine cholesterinartige Verbindung (etwa ein Cholesterinester), resp. ein Gemisch solcher Verbindungen die imprägnierenden Substanzen sein dürften." [After long consideration, I became more and more inclined to the supposition that cholesterol or a cholesterol derivation (such as a cholesterol ester) or a mixture of such, could be the impregnating substance.]

After PFEFFER's and OVERTON's work, not much interest was taken in the peripheral membrane of the protoplast, nor in the tonoplast, which was described first by DE VRIES in 1885. The reason was that investigators were preoccupied with theories explaining protoplasm in terms of colloidal structure. LEPESCHKIN (1924) for example, thought that there were absolutely no membranes and even much later the existence of the outer membrane, the plasmalemma, as called by PLOWE (1931 a), was debated. After a discussion between HÖFLER and WEBER (HÖFLER 1931, WEBER 1932, HÖFLER 1932) the problem of the plasmalemma became of special interest and was investigated by the Viennese school of HÖFLER.

The question of the site of penetration resistance, above all against anelectrolytes and water, became again renewed. HÖFLER emphasized (1931, 1932) that the penetration resistance of the protoplast is apparently the sum of individual resistances $R_p = R_L + R_M + R_T$, *i.e.*, resistance of the protoplast is the sum of the resistances of the plasmalemma, the mesoplasm and the tonoplast. This means that the resistance of the mesoplasm is considered to be of importance, in opposition to the classic concept.

COLLANDER discussed the problem of the site of penetration resistance and writes (1956, p. 211) that the opinions presented in the literature can be somewhat grouped in the following way:

I. R_M is so important that it cannot be neglected in relation to R_L and R_T.

II. R_M is so much smaller than $R_L + R_T$ that it is not of great importance.