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Osmotic gradients in the developing ovule and embryo*

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INTRODUCTION

The present paper summarizes the results of a study which is a continuation of researches made by the author on physico-chemical and physiological processes taking place in the ovule and the embryo of higher plants from early developmental stages following fertilization up to the moment when the ovule attains more or less full maturity (Ryczkowski 1960 a, c; 1962 c; 1964 b; 1965 a, c).

The main object of this study is one of the possible factors inducing polarity in reproductive organs such as a zygote, a proembryo, embryo and ovule, both in lower and higher plants (Wardlaw 1955, 1965). The presence of polarity in these organs was inferred from the results of morphological, anatomical (Maheshwari 1950; Wardlaw 1955), ultrastructural, cyto- and histochemical investigations (Jensen 1963; Pritchard 1964 a).

According to Wardlaw (1963, 1965) the concentration gradient of nutrient substances in the tissues surrounding the egg cell, the zygote, proembryo and embryo is possibly one of the factors inducing polarity and thus influencing the morphogenesis of the developing zygote.

The present work was undertaken with the aim: a) to establish whether it is in the proembryonic and embryonic stage a centripetal osmotic gradient (from the priphery of the ovule towards its centre), and a linear one along the long axis of the ovule (in the direction chalaza → micropyle) in the integumental and nucellar tissues and in the cellular endosperm tissue; b) to find out whether there is in the embryo tissue an osmotic gradient along its long axis during the exponential phase of growth; c) to determine the osmotic value of the embryo at the proembryonic developmental stage.

MATERIAL AND METHODS

Ovules of *Haemanthus Katherinae* have been used as the experimental material. The number of days elapsing from the day of the perianth wilt has been adopted as the measure of the age of organs and tissues.

* This paper is dedicated to Professor Dr F. Górski.

Investigations on the centripetal gradient were carried out in the summer season 1964. They included the determination of the osmotic value 1) of the sap obtained from the integumental and nucellar tissues from whole ovules; 2) of the central vacuolar sap, and later of the sap from the cellular endosperm tissue. Investigations on the linear gradient (chalaza → micropyle) in the above mentioned tissues of the ovule and endosperm were carried out in the summer 1965. They included also the determination of osmotic values of the central vacuolar sap. The procedure used for the extraction of the sap from the central vacuole is described in previous paper (R y c z k o w s k i 1960 a, c).

For the determination of the osmotic value of the sap pressed out from the integumental and nucellar tissues, from the endosperm and embryo the following procedures were elaborated for the isolation of these tissues or organs from the ovule.

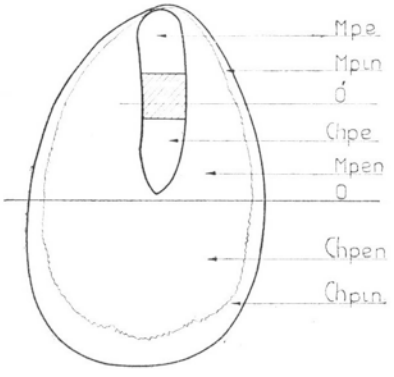

Fig. 1. *Haemanthus Katherinae.* Scheme of longitudinal section of the ovule after the disparition of the central vacuole.

O — plane dividing the ovule into two parts: upper-micropylar and lower-chalazal, *O'* — plane dividing the embryo into two parts: upper-micropylar part with the radicle (*Mpe*) and lower-chalazal part with the shoot apex (*Chpe*), *Mpin* — micropylar part of the integumental and nucellar tissues, *Chpin* — chalazal part of the integumental and nucellar tissues, *Mpen* — micropylar part of the endosperm tissue, *Chpen* — chalazal part of the endosperm tissue.

Integuments + nucellus. After the isolation of the ovules from the fruit and the collection of the vacuolar sap the ovules were cut into two approximatively equal parts. For the sake of brevity the upper, or micropylar part of the tissue in question was denoted by the symbol *Mpin,* whereas, the lower or the chalazal part was designed by *Chpin* (Fig. 1). The rest of the central vacuolar sap and endosperm tissue (or only endosperm tissue in ovules older than 35 days) were removed from the inside of the two parts by means of small sterile pieces of lignine, or pincette and scalpel.

Endosperm. After the central vacuole had been replaced by the

growing endosperm tissue the latter one was easily isolated from the integuments and the rest of the nucellus tissue either by pressing a half of the ovule from outside delicately between two fingers or by means of a small scalpel. The endosperm tissue obtained from the micropylar part of the ovule was denoted by *Mpen* and that from the chalazal part — by *Chpen* (Fig. 1).

Embryo. The part of the integuments from the micropylar half of the ovule, where the upper part of the embryo (the radicle) is adherent to them was delicately removed by means of a scalpel. The ovule was subsequently compressed delicately between two fingers at the micropylar part and through a small aperture made in the integuments the embryo was pressed out. The extracted embryo was dried on a piece of clean filtration paper and cut into two more or less equal parts. In the following the micropylar part of the embryo with the radicle is denoted *Mpe* and the chalazal part of the the embryo with shoot apex — by *Chpe* (Fig. 1). From embryos over 63 days old an middle zone 2—3 mm long was removed (the hatched area in Fig. 1) in order to increase the difference in osmotic values between *Chpe* and *Mpe*.

From these in this way prepared parts (halves) isolated from three types of tissues the sap was obtained in the previously described way (Ryczkowski 1962 c). In the summer season 1964 the sap was pressed out from the integuments + nucellus and endosperm tissue under the pressure of 0,721 kg/mm², whereas, in the summer season 1965 the sap was collected from all three kinds of tissues under the pressure of 1,451 kg/mm². Before the determination of the osmotic value the sap samples were centrifuged for 5 minutes at 3500 rpm. The osmotic value wes determined by means of the thermoelectric method (Ryczkowski 1960 a, c).

In order to reduce the possible effects of the enzymatic activity on various organic compounds and the osmotic value of the examined tissues, the sequence of pressing out the sap from the different parts of the investigated tissues was changed as well as the sequence in the determination of osmotic value of the obtained samples of sap.

The osmotic values of the tissues plotted in Fig. 2 and 3B are mean values of 2—3 measurements, whereas, the sizes of ovules and embryos (Fig. 3A) are means of 3—5 measurements of the lengths and breadths of ovules or embryos.

RESULTS

Central vacuole (summer season 1964). The initial and rapid increase in the osmotic value of the central vacuolar sap observed in 5—20 days old ovules, from 0,175 to 0,290 M, is followed by a drop from 0,290 to 0,200 M in 20—34 days old ovules. The osmotic value of a young

endosperm tissue sap (43—57 days old ovules) decreases slowly from 0,180 to 0,160 M (Fig. 2, curve I).

Integuments + nucellus. The osmotic value of the integumental and nucellar tissues (from the whole ovule) increases from 0,245 to 0,350 M (ovules 5—20 days old; Fig. 2, curve II). This increase is followed by a drop of osmotic value from 0,350 to 0,250 M in 21—57 days old ovules. The osmotic value of the integumental and nucellar tissues sap is higher than that of the central vacuolar sap by 0,075 to 0,100 M.

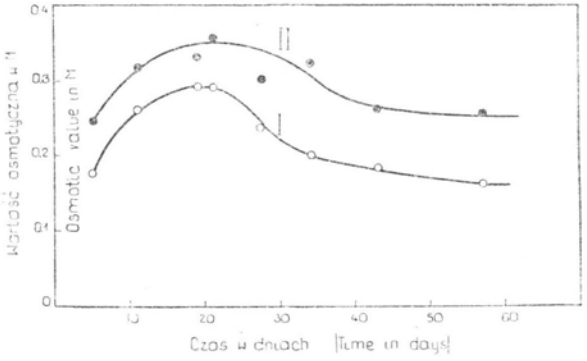

Fig. 2. *Haemanthus Katherinae.* Centripetal (integumental and nucellar tissues → central vacuole) osmotic gradient in the developing ovules. Abscissae: age of the ovules in days counted from the day the perianth wilted.

Curve I — osmotic value of the central vacuolar sap, II — osmotic value of the sap from integumental and nucellar tissues.

Central vacuole (summer season 1965). A rapid increase of the osmotic value of the central vacuolar sap from 0,145 to 0,350 M was observed in ovules at the age of 1—15 days, whereas, in older ones (15—20 to 36 days of age) there is a drop from 0,350 to 0,225 M (Fig. 3B, curve I).

Integuments + nucellus. The osmotic value of the sap obtained from vecuolar sap (Fig. 3B, curve II). A drop of the osmotic value from *Mpin* to 0,150 M (49 days old ovules) is characteristic of ovules over *Mpin* of 11—36 days old ovules is nearly equal to that of the control 36 days old. In 63 days old ovules it equals 0,175 M.

The osmotic value of the sap obtained from *Chpin* increases from 0,400 to about 0,450 M in 11—20 days old ovules, whereas, in older ones (20—49 days old) it drops from 0,450 to 0,270 M and in 63 days old ovules this value is slightly higher and equals 0,300 M (Fig. 3B, curve III).

The difference between the osmotic value of the sap from *Chpin* and *Mpin* in 11—63 days old ovules lies within the limits 0,075—0,125 M; this difference increases with the age of the ovules.

An attempt to determine the osmotic value of the sap from *Mpin* and *Chpin* in 6 days old ovules did not give any decisive result. The difference

between the obtained results lies within the limits of methodical errors; the osmotic value of the sap from *Mpin* was somewhat higher than that of the sap from *Chpin* (Fig. 3B, curve III). The first point on this curve represents the osmotic value of the sap obtained from the integumental and nucellar tissue from whole 1 day old ovules deprived of central vacuolar sap.

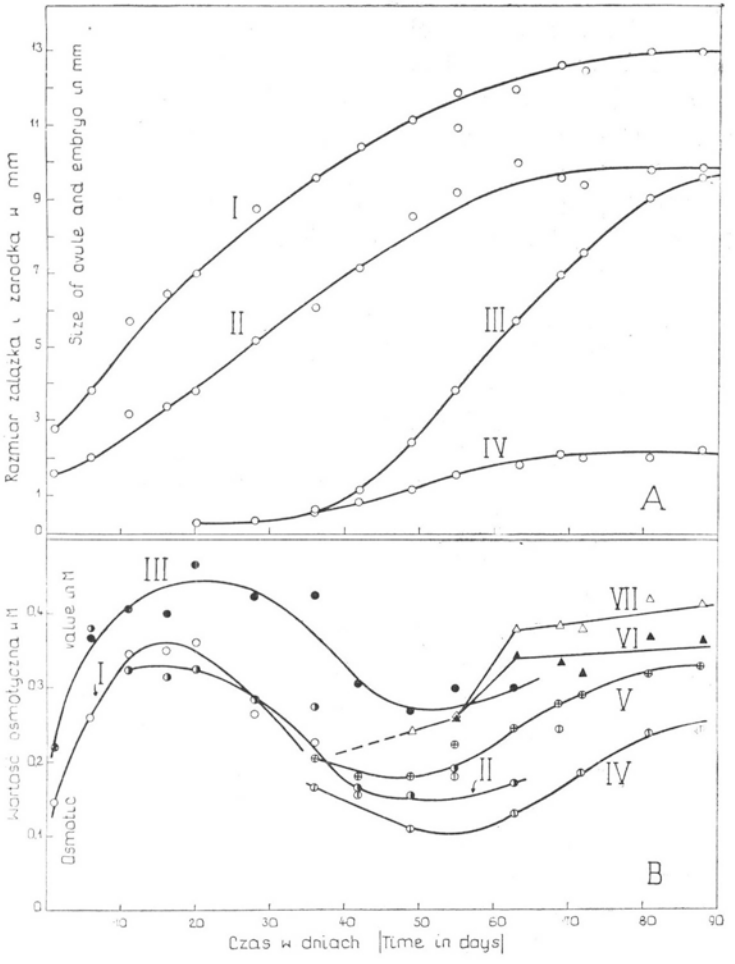

Fig. 3 A—B. *Haemanthus Katherinae.* Linear (chalaza → micropyle) osmotic gradient in the developing ovules and embryos; abscissae: age of the ovules in days counted from the day the perianth wilted.

A. Curve I — length of the ovules, II — breadth of the ovules, III — length of the embryos, IV — breadth of the embryos (in mm). *B.* I — osmotic value of the central vacuolar sap, II — osmotic value of the sap from the micropylar part of the integumental and nucellar tissues (*Mpin*), III — osmotic value of the sap from the chalazal part of the integumental and nucellar tissues (*Chpin*). IV — osmotic value of the sap from the micropylar part of the endosperm tissue (*Mpen*), V — osmotic value of the sap from the chalazal part of the endosperm tissue (*Chpe*), VI — osmotic value of the sap from the micropylar part of the embryo tissue (*Mpe*), VII — osmotic value of the sap from the chalazal part of the embryo tissue (*Chpe*).

Essential changes of the osmotic value of the central vacuolar sap and the sap of integumental and nucellar tissues occur in ovules 1—42 days old during the exponential phase of growth, (Fig. 3A-B) and during the phase of inhibited growth of the embryo, which at this time is in the proembryonic stage — shaped in the form of an irregular sphere. In previous investigations (Ryczkowski 1960 c) it was established that a free nuclear endosperm appears in ovules at the age of 7—9 days after the perianth wilted. In ovules older than 7—9 days cellular endosperm is formed first in the chalazal region and subsequently along the inner layer of the nucellar tissue towards the centre of the central vacuole.

Endosperm. The osmotic value of the sap from the *Mpen* (micropylar part of the endosperm) in 36—49 days old ovules decreases from 0,165 to about 0,110 M, but in still older ovules (49—88 days) it increases from 0,110 to 0,250 M, (Fig. 3B, curve IV).

In the sap obtained from the *Chpen* a small initial drop of the osmotic value from 0,200 to 0,150 M (in 36—49 days old ovules) was observed. This value increased subsequently in 88 days old ovules and attained 0,330 M (Fig. 3B, curve V).

The difference between the osmotic value of the sap from the *Chpen* and *Mpen* varied within the limits 0,050—0,110 M. The smallest difference was found in young endosperm tissue immediately after the disparition of the central vacuole in the ovule. Changes in the sap of the endosperm tissue were observed at the end of the exponential and the begining of the stationary phase of growth of the ovules and during the exponential phase of the embryo growth (Fig. 3A-B).

Embryo. In 49 days old embryos the osmotic value of the sap obtained from the whole embryos equaled 0,245 M (Fig. 3B, curve VI and VII). Older — 55 days old — ovules were characterized by a somewhat higher osmotic value than 49 days old ones. In these ovules differences in osmotic values between *Chpe* and *Mpe* were within the limits of methodical errors. A distinct osmotic gradient was established in embryos 63—88 days old.

Initially the osmotic value of the sap from *Mpe* increases rapidly from 0,260 to 0,340 M (Fig. 3B, curve VI, 55—63 days old embryos), whereas, in still older ones (63—88 days old) it increases very slowly, so that in 88 days old embryos it exceeds the value 0,350 M.

Similar changes in the osmotic value were established for the sap from *Chpe*; however, the obtained values for 63—88 days old embryos were higher by about 0,030 M (Fig. 3B, curve VII) compared with values determined for the sap from *Mpe* of ovules in the some age.

The established changes of the osmotic value in embryo and distinct osmotic gradient between the chalazal and micropylar part of the embryo were found during the exponential phase of growth.

DISCUSSION

The results of the present study on the osmotic value of the tissue sap extracted from the integuments + nucellus, the endosperm, the embryo and the central vacuole are in agreement with previous autor's results (Ryczkowski 1960 a, c; 1962 c; 1965 c).

The discussion of changes of osmotic value of the sap obtained from the integumental and nucellar tissues has been omitted because the general character of their course is analogous to the course of changes in osmotic value of the central vacuolar sap (ovules 1—40 days after the perianth wilting) which were discussed in previous papers (Ryczkowski 1960 a, c; 1964 a; 1965 c).

The author's results are in agreement with Wardlaw's supposition (1955, 1963, 1965) based on morphological and anatomical observations that there is a concentration gradient of nutrient compounds in the tissues surrounding the egg cell, zygote, proembryo and embryo as well as in these organs themselves.

At the proembryonic and embryonic stages there is in the tissues (integuments+nucellus; cellular endosperm) of a *Haemanthus Katherinae* ovule a centripetal osmotic gradient (directed from the periphery towards the centre of the ovule) and a linear gradient (chalaza→micropyle). Both these gradients were found in the tissues of the ovule during its exponential phase of growth (Fig. 2, 3A—B).

A distinct linear osmotic gradient was also found in the embryonic tissue between the chalazal and micropylar part during the exponential phase of growth (Fig. 3A—B).

The osmotic value of the chalazal part of each of the examined tissues (integuments+nucellus; endosperm; embryo) was always higher than that of the micropylar part of the same tissue (11—88 days old ovules).

The smaller the size of the ovule and embryo, and the younger the cellular endosperm tissue the lower the osmotic gradient between the chalazal and micropylar part of the examined tissue (Fig. 2, 3A—B). This finding does not exclude the existence of a gradient in very young and small ovules and embryos. The very small value of this gradient in these organs is doubtlessly a consequence of their small size, leading to rapider equalization of the differences in the osmotic values between the chalazal and micropylar part of the tissue in question. Moreover, the results plotted in Fig. 3A—B do not exclude the possibiliby of the existence of an inverse linear (micropyle → chalaza) osmotic gradient in the integumental and nucellar tissues in very young ovules i.e. at the embryo sac, zygote or at very early developmental stages of the proembryo. This possibility would be in agreement with Wardlaw's (1955, 1963, 1965) and Jensen's (1963, 1965) suggestion. Wardlaw (1965) suggests, however, the possibility of existance of a linear concentration

gradient (chalaza → micropyle) of nutrient compounds at somewhat later developmental stages of the ovule during an intensive growth of the embryo when absorption of nutrients is accelerated.

The results of the author's research on the linear osmotic gradient in ovules of *Haemanthus Katherinae* are in perfect agreement with the results of Zinger (1958). Basing on the results of histo-chemical and anatomical investigations of ovules of several plants species she established that the nutrient compounds are supplied to the ovule by means of a vascular strand which usually ends in the chalaze. It is evident that their concentration will decrease when proceeding from the chalaza to the micropyle because of their absorption by the developing tissues of the ovule.

Previous observations (Ryczkowski 1960c) on the structure of the ovule (*Haemanthus Katherinae*) during its development are also in agreement with the results concerning the linear osmotic gradient (chalaza → micropyle). Basing on these observations it has been established that the tissue of the nucellus and nuclear endosperm is better developed in ovules in the chalazal than in the micropylar part i.e. in the tissue which probably is better supplied with nutrient compounds. The nuclear endosperm tissue contains more nuclei in the chalazal than in the micropylar part. The first cell membranes appearing in the nuclear endosperm are also formed in the chalazal part and subsequently along the inner layer of the nucellar tissue towards the micropyle and the centre of the ovule.

At early developmental stages of the ovule the osmotic value of the proembryo is nearly equal to the osmotic value of the central vacuolar sap.

This conclusion arises from the similitudes of the shapes of the curve representing the changes in osmotic values of the sap from *Mpin* and of the central vacuolar sap (Fig. 3B, curve I and II); it is well known that the proembryo is located in the micropylar part of the ovule between the integumental and nucellar tissues on one side and a thin endosperm layer adjacent to the central vacuolar sap on the other. After the disparition of the central vacuole the osmotic value of the embryo increases concomitantly with its development from the proembryonic stage. This increase proceeds at a higher rate than the increase of osmotic value of the surrounding endosperm tissue (Fig. 3B, curve IV, V, VI, VII); this is in agreement with previous results (Ryczkowski 1962c).

The elongation of the embryo proceeds from the micropylar to the chalazal end, i.e. in a direction opposite to that of the linear gradient of osmotic value in the tissues of the ovule.

The higher osmotic value of the sap pressed out from the embryo (in its exponential phase of growth) in comparison with the osmotic

value of the sap from the endosperm tissue (at the end of the exponential phase of growth of the ovule) may be ascribed to two causes:

a) The first is a shifting of developmental processes in relation to each other, more explicitely a shifting of biochemical processes in the endosperm and embryo tissue. In this case the synthesis of starch and proteins from compounds of low molecular weight would be more intensive in the endosperm tissue than in the tissue of the embryo. In turn this would lead to a lower osmotic value of the endosperm tissue in comparison with the osmotic value of the embryo tissue.

Kolobkova's (1958) data were used to calculate the changes of the ratio of protein nitrogen to amino nitrogen during the four developmental stages of maize grain. It was found that this ratio increased by about 20 times in the endosperm tissue, whereas in the embryo tissue less than 7 times. A similar calculation (based on data by Rijven and Cohen 1961) of the ratio of protein nitrogen to soluble nitrogen in the endosperm and embryo of wheat, 24 to 36 days after pollination, showed that this ratio increased gradually in the endosperm tissue but remaind more or less constant in the embryo-tissue. Ingle et al. (1965) established basing on the analitical results made at determined developmental stages of corn grain that the amounts of such compounds as RNA, DNA, soluble nitrogen, amino acids, sugars expressed in mg/part decreased in the endosperm tissue and increased in the embryo tissue. A drop of soluble nitrogen compounds in the endosperm tissue was ascribed to an intensive protein synthesis in this tissue. Ingle et al. (1965) did not determine the content of starch. It is known, however, that a decrease of sugar content in grains of various plant species results from an intensive synthesis of starch in these grains (McKee 1955; Akazawa et al. 1964).

b) It may be that at this developmental stage of the ovule the embryo is first supplied with nutrient compounds in comparison with the endosperm tissue, and moreover with greater amounts.

In dicotyledonous plant (*Aesculus pavia*) changes of the osmotic value of the sap from embryos in the exponential phase of growth show slight differences in comparison with monocotyledonous one. The osmotic value remains on a more or less constant level for a long period of time and subsequently drops rapidly at the final phase of the disparition of the central vacuole (Ryczkowski 1962c).

The results of the author's investigations on the osmotic value of the embryo sap are not in disagreement with those obtained by Rietsema et al. (1953a, 1955) in their study on embryos of *Datura stramonium* cultured *in vitro*. They found that according to the developmental stage the embryos required different concentrations of sucrose. Thus the pre-heart stage (size 0,1 mm) requires 8—12% solution of sucrose; late heart stage (0,2 mm) — 4%; the early torpedo stage (1 mm) — 1%;

the torpedo stage (2 mm) — 0,1%, and almost mature embryos practically did not need any sugar for growth. It is most probable that the maximum osmotic value of embryos during their development occurs at the pre-heart stages, similarly as it was found for 15—20 days old ovules of *Haemanthus Katherinae*. If Rietsema et al. (1953a, 1955) had included in their research embryos still younger than the pre-heart stage most probably they would have found that these require lower sucrose concentrations than the embryos in the pre-heart stage.

SUMMARY

By means of a thermoelectric method (Ryczkowski 1960a, c) a study has been carried out on the osmotic gradient in various tissues of the ovule and embryo of *Haemanthus Katherinae* during the exponential phase of growth. Besides, the osmotic value of the central vacuolar sap has been determined.

A centripetal osmotic gradient directed from the periphery of the ovule towards its centre and a linear gradient in the direction chalaza → micropyle have been established in the tissues of the ovule (integuments+nucellus; cellular endosperm) at the proembryonic and embryonic stages of development. A distinct linear gradient (chalaza → micropyle) has also been found in the embryo tissue (Fig. 2, 3A—B).

In the tissues of the ovule the elongation of the embryo proceeds in a direction that is opposite to the direction of the osmotic linear gradient.

The osmotic value of the proembryo is equal or nearly equal to the osmotic value of the central vacuolar sap and changes during the development of the ovule and embryo.

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REFERENCES

Akazawa T., Minamikawa T. and Murata T., 1964, Enzymatic mechanism synthesis in ripening rice grains, Plant Physiol. 39:371—378.

Ingle I., Beitz D. and Hageman R. H., 1965, Changes in composition during development and maturation of maize seeds, Plant Physiol. 40:835—839.

Jensen W. A., 1963, Cell development during plant embryogenesis. Meristems and differentiation, Brookhaven symposia in Biology USA.

Jensen W. A., 1965, The ultrastructure and histochemistry of the synergids of cotton, Amer. J. Bot. 52:328—356.

Kolobkova E. V., 1958, Nitrogen metabolism in ripening seeds of *Zea mays*, Dokl. Akad. Nauk. SSSR. 120:907—908.

Maheshwari P., 1950, An introduction of the embryology of angiosperms, New York, Toronto, London.

McKee H. S., Robertson R. N. and Lee. J. B., 1955, Physiology of pea fruits, Aust. J. Biol. Sci. 8:137—163.

Pritchard H. N., 1964a, A cytochemical study of embryo sac development in *Stellaria media*, Amer. J. Bot. 51:371—378.

Rietsema J., Satina S. and Blakeslee A. F., 1953a. The effect of sucrose on the growth of *Datura stramonium* embryos in vitro, Amer. J. Bot. 40:538—545.

Rietsema J., Blondel B., Satina S. and Blakeslee A. F., 1955, Studies on ovule and embryo growth in *Datura*. I. A growth analysis, Amer. J. Bot. 42:449—455.

Rijven A. H. G. C. and Cohen R., 1961, Distribution of growth and enzyme activity in the developing grain of wheat, Aust. J. Biol. Sci. 14:525—566.

Ryczkowski M., 1960a, Obsevations on the osmotic value of the central vacuole sap in *Haemanthus Katherinae* Bak. ovule, Bull. Acad. Polon. Sci., Ser. sci. biol. 8:143—148.

Ryczkowski M., 1960c, Changes of the osmotic value during the development of the ovule, Planta 55:343—356.

Ryczkowski M., 1962c, Changes in the osmotic value of the sap from embryos, the central vacuole and the cellular endosperm during the development of the ovules, Bull Acad. Polon. Sci., Ser., sci. biol. 9:375—380.

Ryczkowski M., 1964a, Physico-chemical properties of the central vacuolar sap in developing ovules (mono- and dicotyledonous plants). [In:] Linskens H. F. (ed), Pollen physiology and fertilization, p. 17—25, Amsterdam.

Ryczkowski M., 1965a, Physical properties of the sap surrounding the embryo in developing ovules, Exptl. Cell Res. 38:120—127.

Ryczkowski M., 1964 a, Physico-chemical properties of the central vacuolar sap differentiation of the egg cell in developing ovules *Cycas revoluta (Gymnospermae)*, Bull. Acad. Polon. Sci., Ser. sci. biol. 13:557—559.

Wardlaw C. W., 1955, Embryogenesis in plants, London, New York.

Wardlaw C. W., 1963 Plant embryos as reaction system. [In:] Maheshwari P. (ed.), Recent advances in embryology of angiosperms, p. 355—360, Delhi.

Wardlaw C. W., 1965, Physiology of embryonic development in cormophytes. [In:] Rhuland (ed.), Encyclopedia of Plant Physiology, 15/1:424—442. Berlin, Heidelberg, New York.

Zinger N. V., 1958, The seed, its development and physiological properties, Moscow.

Gradient wartości osmotycznej w rozwijających się zalążkach i zarodkach

Streszczenie

Przy pomocy metody termoelektrycznej (Ryczkowski 1960a, c) przeprowadzono badania nad gradientem osmotycznym w różnych tkankach zalążka (*Haemanthus Katherinae*) i zarodka podczas wykładniczej fazy ich wzrostu, ponadto przeprowadzono oznaczenia wartości osmotycznej soku centralnej wakuoli.

Stwierdzono, że na stadium prozarodka i zarodka właściwego w tkankach (integumentów+nucellus; endospermu) zalążka istnieje gradient osmotyczny do-

środkowy (od zewnątrz zalążka do jego środka), oraz gradient liniowy (chalaza → micropyle). Również w tkance zarodka stwierdzono wyraźny gradient liniowy (chalaza → micropyle), Ryc. 2, 3A—B.

Wydłużanie się zarodka następuje w kierunku przeciwnym do kierunku istniejącego liniowego gradientu wartości osmotycznej w tkankach zalążka.

Wartość osmotyczna prozarodka jest taka sama, względnie bardzo zbliżona do wartości osmotycznej soku centralnej wakuoli i zmienia się w trakcie rozwoju zalążka i zarodka.