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# Sugars and osmotic value of the sap surrounding the embryo in developing ovules (dicotyledonous perennial plants)

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In his previous papers (1960a,b,c, 1961 and 1962a) the author examined some changes of the physico-chemical properties of the central vacuolar sap occurring during the development of the ovules. These investigations were carried out on ovules of monocotyledonous, annual plants. In the ovules of these plants the developing endosperm tissue is adjacent to the central vacuolar sap whereas the contact of the embryo with the central vacuolar sap is an indirect one through the medium of this tissue. Concomitantly with the growth and development of the ovule the endosperm tissue gradually replaces the central vacuole till its complete disparition.

The present paper is a continuation of the investigations carried out on the physico-chemical properties of the central vacuolar sap in ovules of dicotyledonous plants, in which both the embryo and the endosperm are adjacent to the central vacuolar sap.

The present investigations have been carried out with the aim to determine: a) the concentration of sugars (reducing, total and non-reducing ones) in the central vacuolar sap during the development of the ovules, b) the osmotic value of the central vacuolar sap, c) the concentration of osmotically active substances other than sugars.

## MATERIAL AND METHOD

Ovules of *Aesculus pavia* and *Aesculus glabra* provided the experimental material. The size of the ovules and the size of their embryos were the adopted criteria of growth and development of the ovules. The size of ovules was determined by measuring their breadth and length whereas in embryos the length of their convex curvature and the breadth of the cotyledons in the largest place (R y c z k o w s k i 1962b,c) were measured. The material for analysis was collected between 7 and 8 o'clock a.m.

Sugars were determined by means of the S o m o g y i (1945) micro--method modified by W i l l i s and Y e m m (1955). The extracted sap of the central vacuole 0.1 ml was added to 1 ml absolute alcohol. The sample was then evaporated in a vacuum to dry residue which was then dissolved in twice distilled water with addition of aluminium hydroxyd cream. The solution was brought to a constant volume. A part of this solution was filtered through a Schott G 4 filter; 0.5 to 1 ml of this clear solution was then taken for analysis. The results of sugar and osmotic value determination are given in mg/ml or in moles per liter (M/ltr). A full description of the method of sugar determination was presented in the author's paper 1962a. Measurements were performed in summer 1961 and 1962.

## RESULTS

*Aesculus pavia.*

Sugars. The concentration of reducing sugars increases in the central vacuolar sap from 20—27 mg/ml concomitantly with the increase of the breadth of the ovules from 5.3 to about 8 mm; (Fig. 1, curve I) and then drops from 27 to 0 mg/ml with the further growth of the ovule

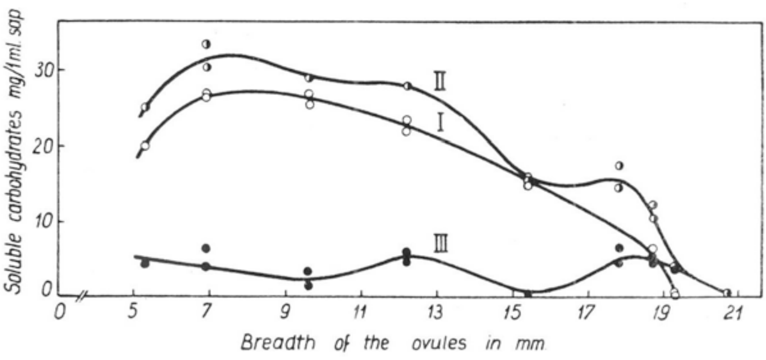


Fig. 1. *Aesculus pavia.* Changes in the concentrations (mg/ml) of sugars in the sap surrounding the embryo in developing ovules; abscissae: breadth of the ovules in mm.

Curve I — reducing sugars, II — all soluble sugars after hydrolysis, III — non-reducing sugars.

(breadth from 8—19.3 mm). The concentration of total sugars soluble in the central vacuolar sap increases from 25 to 32 mg/ml (5.3 to 8 mm broad ovules, curve II); then it drops to about 15 mg/ml sap with the further increase of the ovules to 15 mm breadth. The concentration of total sugars maintains on a constant level of 15 mg/ml in 15.4 to 17.8 mm broad ovules. A rapid drop of the concentration of total sugars in the central vacuolar sap from 15—0 mg/ml is observed in older ovules of 17.8 to 20.7 mm breadth. The concentration of non-reducing sugars in the central vacuolar sap oscillates within the limits of 0 to 5 mg/ml (Fig. 1, curve III).

Osmotic value. Changes of osmotic value of the central vacuolar sap during the development of the ovules are shown in Fig. 2, curve I (from the year 1961) and curve II (from the year 1962). Osmotic value increases from 0.40 to 0.49 M (curve I) in 5.6 to 9.0 mm broad ovules and then drops from 0.49 to about 0.3 M. with the further growth of the ovules into breadth.

Measurements of the osmotic value of the central vacuolar sap performed in 1962 (curve II) differ greatly from those performed in 1961. A small increase of the osmotic value from 0.43 to 0.46 M is characteristic of young ovules (5.0 to 8.0 mm broad). A drop of osmotic value from 0.46 to 0.39 M and its subsequent increase from 0.39 to 0.445 M is observed in older ovules (8.0 to 11.9 and 11.9 to 16.9 mm broad ovules respectively). A rapid drop of osmotic value of the central vacuolar sap to about 0.35 M is characteristic of older ovules (17.0—19.2 mm broad).

Curve I U.c. (undetermined compounds) represents the difference between curve I and III which results from expressing the content of reducing sugars and non-reducing sugars in terms of osmotic value (moles per liter). As it can be seen from curve I U.c. the concentration of

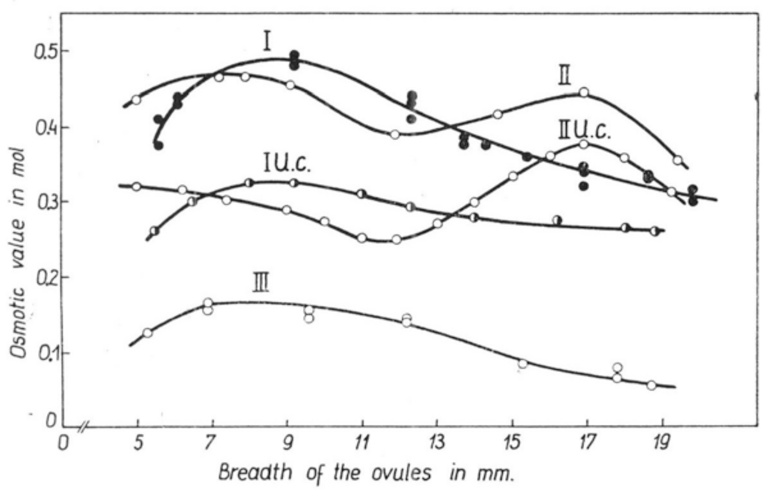


Fig. 2. *Aesculus pavia.* Changes in the osmotic value of the sap surrounding the embryo in developing ovules.

Curve I — osmotic value of the sap (determined 1961), II — osmotic value (1962), III — osmotic value of the sap calculated from the concentrations of reducing and non reducing sugars of the sap surrounding the embryo, curve I.U.c. (undetermined compounds) in the sap i.e. difference between curves I and III, curve II.U.c. — difference between curves II and III.

osmotically active substances (without sugars) increases from 0.26 to 0.33 M in young ovules (5.5 to 9.0 mm broad) and gradually decreases from 0.33 to 0.26 M in older (9.6 to 18.8 mm broad) ovules.

The concentration of osmotically active substances (without sugars) shown by the curve II U.c. (difference between curve II and III) differs greatly from the concentration of those substances presented by curve I U.c. The concentration of osmotically active substances decreases from 0.32 to 0.25 M in young ovules (5.0—11.9 mm broad) and subsequently increases from 0.25 to 0.38 M in older ones (11.9 to 16.9 mm broad). A drop of osmotic value of the central vacuolar sap from 0.38 to 0.31 M is characteristic of still older — 17.0 to 19.2 mm broad — ovules.

*Aesculus glabra.*

Sugars. The concentration of different groups of sugars, soluble in the central vacuolar sap, undergoes during the development of the ovules of this plant (the Table) similar changes to those which were

Table 1

*Aesculus glabra.* Changes in the concentrations (mg/ml) of sugars and osmotic value (mol/liter) in the sap surrounding the embryo in dependence on the sizes of ovules and embryos.

| Size of ovules in mm | Size of embryos in mm | Free reducing sugars mg/ml | Total soluble carbohydrates (after hydrolysis) mg/ml | Non-reducing sugars mg/ml | Size of ovules in mm | Osmotic value in mol |
|---|---|---|---|---|---|---|
| 6.2 × 4.8 | | 26.50<br>26.25 | 30.50<br>32.00 | 4.00<br>5.75 | 5.3 × 4.2 | 0.455 |
| 8.3 × 6.1 | | 29.00<br>29.00 | 40.50<br>41.25 | 11.50<br>12.25 | 6.6 × 5.2 | 0.559 |
| 10.8 × 8.0 | | 41.00<br>40.00 | 41.00<br>40.00 | 00.00<br>00.00 | 8.0 × 6.0 | 0.544 |
| 12.0 – 9.3 | | 32.50<br>32.50 | 35.25<br>34.25 | 2.75<br>1.75 | 8.3 × 6.5 | 0.504 |
| 13.9 × 11.5 | 4.4 × 2.1 | 26.00<br>26.00 | 35.50<br>33.00 | 9.50<br>7.00 | 10.8 × 7.6 | 0.489 |
| 17.3 × 14.4 | 8.9 × 3.1 | 20.00<br>19.00 | 23.75<br>25.00 | 3.75<br>6.00 | 12.9 × 9.4 | 0.473 |
| 19.3 × 14.9 | 11.9 × 4.1 | 18.50<br>19.50 | 23.00<br>20.50 | 4.50<br>1.00 | 16.4 × 12.6 | 0.476 |
| 18.8 × 14.9 | 24.9 × 10.1 | 1.75<br>1.50 | 12.00<br>11.00 | 10.25<br>9.50 | 16.0 × 12.6 | 0.436 |
| | | | | | 18.4 × 16.0 | 0.345 |

established in the central vacuolar sap of *A. pavia* ovules. Absolute values of concentrations of all the three groups of sugars presented in the Table (*A. glabra*) are higher than the analogous values of concentrations found for the central vacuolar sap of *A. pavia* ovules. Besides, an initial increase of non-reducing sugars was established in the central vacuolar sap of 4.8—6.1 mm broad ovules of *A. glabra*. In older ones (14.9 mm broad) a remarkable transformation of reducing sugars into non-reducing ones was observed. In the central vacuolar sap of *A. pavia* ovules this phenomenon occurred at a very low degree.

Osmotic value. The osmotic value of the central vacuolar sap in ovules of *A. glabra* changes during their development in an analogous way as in ovules of *A. pavia* i.e. it increases in young ovules (4.2—6 mm broad) from 0.455 to 0.544 M and in older ones (6.5 to 16.0 mm broad) it decreases to 0.345 M.

Neither two maxima of osmotic value nor a minimum between them were established in the ovules of this plant.

The calculation of the concentration of osmotically active substances (without sugars) revealed that during the development of the ovules the concentration changes in a way similar to that of the osmotically active substances (without sugars) in the central vacuolar sap in *A. pavia* ovules (1962).

## DISCUSSION

The author explains the increase of the osmotically active substances in the central vacuolar sap in *A. pavia* and *A. glabra* ovules in their early developmental stages in an analogous way as in monocotyledonous plants (1960 a, c, 1962 a), it means by an inflow of osmotically active compounds from vegetative organs into the ovule with water. At this developmental stage of the ovule the inflow of osmotically active compounds is more intensive than that of the water inflow, whereas the decrease of all osmotically active substances is mainly due to their dilution (*A. pavia* ovules 9—15—16 mm broad, *A. glabra* ovules 8—12 mm broad). Measurements of the ovules and their embryos revealed that the embryo in a 19.4 × 15.4 mm large *A. pavia* ovule is about 5.1 × 2.5 mm large and fills out the lateral cavity in the embryo sack and the central vacuole attains its maximum size. At this and at earlier stages the developing embryo takes up the nutrient compounds, doubtlessly, not only from the tissue of the nucellus but also from the central vacuolar sap. In older ovules (*A. pavia* 17—20 mm broad, *A. glabra* 12—16 mm broad) the decrease of the osmotically active substances (sugars, amino-acids, mineral salts) is caused by an intensive growth of the embryo, and specially of its cotyledons (the Table; Ryczkowski 1962 c).

The results of the determination of the osmotic value and the concentration of sugars in the central vacuolar sap are in agreement with analogous results obtained for the central vacuolar sap of ovules of monocotyledonous plants. They are also in agreement with the results obtained by other investigators who determined sugars, amino acids, nonvolatile organic acids and phosphorus compounds in coconut water. Tulecke *et al.* (1961) established that the content of glucose and fructose in coconut water in the young green stage (400 ml coconut water) equaled 8.23 mg/ml, 12.5 mg/ml in the mature green stage (253 ml water) and 4.97 mg/ml in the mature fresh stage (192 ml water). The content of saccharose was: 0.93; 9.18; 8.9 mg per mililiter coconut water respectively. The amounts of nonvolatile organic acids determined at the same stages of development as sugars were: 12,56; 35,98; 13,33 Meq/ml coconut water respectively. Baptist (1963) who determined the amino-acids in coconut water established that the total amounts of free amino-acids were: 19; 41; 318; 685 μmoles per 100 g liquid endosperm in 4—5; 6—7; 8—9; 10—12 months old ovules respectively. He also found that the total amounts of free amino-acids in solid endosperm at the stage of 6—7, 8—9, 10—11 and 12 months were: 980, 1380, 1160, and 1169 μmoles per 100 g solid endosperm respectively. The phosphorus compounds in the coconut water behave during the development of the nut in a similar way as the amino-acids (Wilson and Cutter jr. 1952).

The difference between the above results and the osmotic value and sugar content in the central vacuolar sap (determined by the author) is due to the fact that the content of free amino-acids (Tulecke *et al.* 1961; Baptist 1963) and inorganic phosphorus compounds (Wilson and Cutter jr. 1952) do not decrease in the central vacuolar sap in the final developmental stage of the coconut. This phenomenon may be connected with the fact that the withdrawal of these compounds from the coconut water to the developing endosperm tissue was compensated by a concomitant decrease of water content (Wilson and Cutter jr. 1952). The fact that Tulecke *et al.* (1961) and Baptist (1963) did not investigate the last phase of the nut development may also be responsible for it. Experiments were ended at the stage when the coconut water content equaled 150—190 ml in a nut. The presence of a high content of the same amino-acids in solid endosperm and in coconut water points to the fact that they have been taken up from the latter.

The above results of the author are in agreement with the determinations on the nitrogen compounds carried out by a number of investigators and arrayed by McKee (1958). It results from the data given by McKee (1958) that in seeds of *Vicia faba*, *Pisum sativum* and wheat the amount of non-protein nitrogen or soluble nitrogen increases in their early stages to a determined value and subsequently drops with the growth and development of the seeds. On the other hand the amount of protein compounds is lower at the early developmental stages of the seed and gradually increases up till the moment the seed has ripened.

The latest investigations by R i j v e n (1961) K o l o b k o v a (1961) corroborate the above results.

The autor's supposition is that — as in monocotyledonous plants — amino-acids, phosphorus compounds and such ions as K and Ca are among the most important components of the undetermined compounds (curve U.c. fig. 2). This hypothesis is corroborated, as well, by the results of other investigators, especially by D e l e a n o and B o r d e i a n u (1933) and T a m m e s (1959).

The results concerning the concentration of sugars in the central vacuolar sap in ovules of dicotyledonous plants differ from the results obtained by other investigators, for whole seeds in a similar way as it has been established for the central vacuolar sap of the ovules of monocotyledonous plants (R y c z k o w s k i 1962 a); this problem will be discussed in an other paper.

Due to different climatic conditions in summer 1961 and 1962 great differences in the changes of osmotic value of the central vacuolar sap in ovules of *A. pavia* were recorded. Measurements of temperature and rainfall performed in summer 1961 and 1962 on the territory of the Botanical Garden in Cracow (where the material for analysis was collected) showed that the first maximum of osmotic value in 1962 was observed in the first ten days of July when the mean temperature of air at 7.30 a.m. was 11.7°C; whereas the maximum of osmotic value of the central vacuolar sap in 1961 occurred in the last decade of June when the mean temperature of the air at 7.30 a.m. was 16.7°C. The minimum of osmotic value between the maxima fell on the second decade of July (1962) when the rainfall equaled 118,8 mm. Such an amount of rainfall was not observed in any other decade of the summer months (June, July, August) either in 1961 or in 1962. The further increase of osmotic value in ovules of *A. pavia* (12—17 mm broad ovules, 1962) fell on the last decade of July and the first decade of August, i.e. on a period of sunny and warm weather, characterised by a mean temperature over 17°C (at 7.30 a.m.) and small rainfalls. It results from curve II U.c. fig. 2 that the second increase of the osmotic value in ovules of *A. pavia* was most probably conditioned by an increase of concentration of mineral compounds in the central vacuolar sap because the concentration of sugars in the central vacuolar sap decreases at this developmental stage of the ovules. In ovules of *A. glabra* no minimum of osmotic value was observed at the same time, as in ovules of *A. pavia*. It maintained at a constant level (the Table, underlined results). This is most probably connected with the growing of *A. pavia* on flat ground where the water circulation is slow; whereas *A. glabra* grows on a rather raised (elevated) territory with a better water circulation.

Unfavourable climatic conditions in summer 1962 influenced unfavorably the physico-chemical properties of the central vacuolar sap and their changes during the development of the ovules. The character of the osmotic changes was, according to the author's opinion, not quite normal. This influenced, of course, the growth and development of the ovules in an inhibiting way. Embryos, small in size, with corrugated cotyledons, of which one was as a rule worse developed than the other or even completely undeveloped, were often found.

On the grounds of thus far performed investigations on the physico-chemical properties of the central vacuolar sap in mono — (Ryczkowski 1960 a, c, 1962 a) and dicotyledonous plants both similarities and differences have been found to appear among these changes. It has been established that the osmotic value of the central vacuolar sap increases in young ovules of both these groups and decreases in older ones. The concentration of sugars in the central vacuolar sap changes in an analogous way as the osmotic value. In older ovules of both these plant groups reducing sugars are transformed into non-reducing sugars and these are taken up by the developing endosperm tissue or by the embryo (Ryczkowski 1962 b, c) where they, at least to a certain extent, undergo most probably a further transformation into starch (Evans 1941, Danielson 1956).

The differences between the changes of physico-chemical properties of the central vacuolar sap in both groups of plants refer chiefly to absolute values shown by different groups of compounds and to the character of their changes during the development of the ovules. And so e.g. the non reducing in the central vacuolar sap in young ovules (monocotyledonous plants) increases with their development to a determined maximum value *Clivia* sp. 47 mg/ml, *Haemanthus Katharinae* about 30 mg/ml) and drops subsequently. In dicotyledonous plants the concentration of reducing sugars oscillates within the limits 0—5 mg/ml (*A. pavia* or from 0—12 mg/ml (*A. glabra*). On the other hand, the undetermined osmotically active substances (U.c.) in the central vacuolar sap in ovules of *A. pavia* are considerably higher than in the central vacuolar sap of ovules of *Clivia* sp. (Ryczkowski 1962 a). The shape of the curves which present the changes in this group of compounds is different for each of these two plants.

The physico-chemical differences shown by the central vacuolar sap are partly attributable to the differences in the structure, in the size of the ovules as well as to the fact that mono and dicotyledonous plants belong to different systematic groups.

## SUMMARY AND CONCLUSIONS

1. Measurements of osmotic value and sugar concentration (reducing, non-reducing, total sugars) in the central vacuolar sap in ovules of dicotyledonous plants *Aesculus pavia* and *Aesculus glabra* have been performed.

2. Osmotic value has been determined according to the thermo-electric method (Ryczkowski 1960a and c), sugars — according to the Somogyi (1945) micro-method modified by Willis and Yemm (1955), (Ryczkowski 1962 a).

3. It has been established that the osmotic value and sugar concentration (reducing, non-reducing and total sugars) increase to a determined maximum in young ovules of both these plants; in older ovules both the osmotic value and sugar concentration decrease considerably.

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## *Cukry i wartość osmotyczna soku wakuoli rozwijających się zalążków Aesculus pavia i A. glabra*

Streszczenie

1. Wykonano pomiary wartości osmotycznej i stężenia cukrów (redukujących, nie redukujących, globalne) w soku centralnej wakuoli podczas rozwoju zalążków u roślin dwuliściennych, *Aesculus pavia* i *Aesculus glabra*.

2. Wartość osmotyczną oznaczono metodą termoelektryczną (Ryczkowski 1960 a i c), cukry — zmodyfikowaną przez Willis i Yemm (1955) mikrometodą Somogyi (1945); Ryczkowski (1962 a).

3. Stwierdzono, że u zalążków młodych obu roślin wartość osmotyczna i stężenie cukrów (redukujących, nie redukujących, globalne) podwyższa się do określonego maksimum, a u zalążków starszych zarówno wartość osmotyczna, jak i stężenie cukrów, znacznie się obniża.

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