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Anatomy of the fruit and seeds of *Viscum*

by

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(With 1 plate.)

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Germination of *Viscum album* is characterized by a number of special features. It has been known for a long time that the seeds of this plant cannot be germinated in the dark.[1] Studies by Wiesner[2] have shown that a not inconsiderable intensity of light is required, especially at the start of germination, and that the seeds only germinate after a long period of rest. So far, it has not been possible to germinate the seeds of this plant before the end of March or the beginning of April.

Furthermore, Wiesner[3] has shown that the seeds of *Viscum album* can germinate completely free from the slime of the berry without the addition of water on a dry substrate. Yes, even in the desiccator with seeds placed over sulfuric acid, seedlings develop hypocotyls up to a certain limit.

"The force with which this water, which is indispensable for the germination of the mistletoe seeds, is retained by the tissues of the mistletoe, may be seen from the fact that weak germination occurs even when the seeds are in the desiccator."[4]

So while the seeds of *Viscum album* in ripe condition contain the water in their tissues they need to germinate, the seeds of the tropical *Viscum* species cannot be germinated without the addition of water, even in an atmosphere that is almost saturated with water vapor. The seeds of the tropical *Viscum* species also germinate without going through a period of rest; they also germinate in complete darkness.[5]

It is highly probable from the outset that the strange peculiarities that emerge when *Viscum album* germinates are adaptations to the external vegetation conditions and have been proven by Wiesner [6] in the most definite way.

Among other things, he showed that during the period for us in which *Viscum album* sprouts, there is often a rainless period that is so long that the amount of water accumulated in the seed and stubbornly retained is necessary for germination.

At the instigation of the Mr. Hofrathes J. Wiesner, I undertook to investigate the peculiarities of the anatomical structure that are associated with this exceptional transpirational protection in context, whereby, of course, consideration was also given to the analogous relationships of the seeds of tropical *Viscum* species. Before I go into my own research, I would like to make a few morphological comments about the mistletoe..

The development of mistletoe has been the subject of older and newer studies by many botanists, such as Decaisne,[7] Meyen,[8] Schacht,[9] Karsten,[10] Treviranus,[11] Hofmeister,[12] van Tieghem,[13] Treub,[14] Jost[15] et al. So you are very clear about that now.

I will try shortly to reproduce the characteristic moments of flowering of the mistletoe.

The female flower has two bipartite whorls of perigone leaves. In the middle there are two carpels, which fuse with the receptacle in such a way that they represent a homogeneous mass. The cells around the bottom of the former fissure that formed the two carpels incurs a division. The only exception are a few cells that are notable for their size and dark content. These are the embryo sac mother cells. They are usually laid out in a number of seven or nine. The nucleus of the embryo sac mother cells divides later and a transverse wall appears. The majority of these cells thus divided remain at this stage; at most two or three continue to develop: the lower of the two sister cells enlarges and develops into an embryo sac.

An embryo develops in each embryo sac and, depending on the number of embryo sacs, the so-called seed has one, two or three embryos.

In *Viscum* does not form ovules, even the placenta is not developed, but the embryo sacs arise directly from the tissue of the axis. Each embryo sac corresponds to one seed, which has not been separated. One cannot speak of seed coat or seed skin here, since these would have to form from the integuments of the ovule. With *Viscum*, however, as already mentioned, ovule formation does not occur.

The core of the berry of *Viscum album* is usually called a seed, which, of course, is not correct from a strictly morphological point of view. But if you only look at the berry kernels [core, pip] from a physiological point of view, so they can be described as seeds, the false berries of the mistletoe as fruits.

The ripe berries of *Viscum album* are almost pure white in color and the top of the berries still show traces of the dried stigma and the perigone leaves.

The size of the *Viscum* berries varies considerably. They consist of the fleshy casing and the kernel, the so-called seed, which in turn includes one, two or three embryos.

The fleshy shell consists of two layers, the inner of which is so slimy that it is a homogeneous, slimy mass for the unarmed eye. The outer, as well as the inner, the so-called viscin layer, develops from the tissue of the cup-shaped floral axis.

If you look at this slimy, strongly adhesive mass under the microscope, you can see that it mainly consists of elongated cells with spiral thickening. These cells contain abundant protoplasm, as well as a small amount of starch granules. There are also crystals of oxalic acid lime in the slime, in larger quantities. These are well-developed, smaller and larger individual crystals. Crystalline glands are very rarely found in the viscin slime.

If you take the berry as carefully as possible, remove the slime without pulling it into threads, and treat it with chlorozinc iodine, it will turn purple after a while, and only marginally. The cells now appear more clearly there: their thickenings are colored, while the outer membrane layer is indistinguishable (Fig. 7). The reagent penetrates the slime very poorly. With half-dried slime, the coloring proceeds a little faster, but still only a small part of it stains.

The viscine slime has the property of being pulled out into threads which dry quickly in the air.

If you treat slime extracted from threads with chlorozinc iodine, it quickly turns purple. If the cover glass is pressed lightly, the threads disintegrate into even finer, parallel ones. Even these are not the same thickness. Each thread now corresponds to a cell and after some cells are more, some less expanded, they appear to have different thicknesses. The spiral thickenings are visible when the lines are less extended, which is not the case when the lines are extended. Even on one and the same cell one can see how half of them pulled out into a long, thin thread, while the other suffered only a slight change.

If you let iodine tincture + sulfuric acid act on the slime pulled out in the threads, it turns blue.

With corallin soda it turns red. With ruthenium red [16] (ruthenium sesquichloride), it turns pale red, i.e. as far as the pure cotton.

It swells in potash lye, but does not dissolve.

Hydrochloric acid has no effect. The viscin slime dissolves in sulfuric acid and copper oxide ammonia. It is insoluble in cold and hot water. It behaves just like ordinary cellulose against the reagents mentioned.

If you harden the berry, gradually in weak, and finally in absolute alcohol, you can make cuts through the fleshy casing without exposing the tissue to excessive shrinkage. On these cuts you can already see the color that the fleshy casing consists of two layers. The outer is dirty white, while the inner is snow white in color.

The epidermis of the outer layer is thickened and cuticular. The cells are parenchymatous and very thin-walled. They have a large cell nucleus and a small amount of starch and chlorophyll grains. The bundles of vessels run at a certain distance from the surface.

These cells of the outer layer of the fleshy casing are also quite mucilaginous and completely mucilaginous are those that adjoin the viscine layer. The latter consists of the elements mentioned above, which are arranged radially against the seed. If you add a drop of water to such a cut, the cells swell so much that sometimes they are no longer visible.

Already in the older literature there is some information about the mistletoe slime. Treviranus [17] believed that the adhesive material "viscine" was in elongated, colorless tubes, which were radiating from all points of the circumference against the "egg".

H. Schacht [18] states about the viscin that it is not a special compound, but first forms a decomposition product of the cellulose of the wall of the cells that surround the mistletoe seed. He also speaks of the spiral thickening of the cells.

Karsten's[19] views are of historical value only.

The viscin layer serves to attach the mistletoe fruits to the host branches. The seed is surrounded by a thin, white, shiny silver skin, the so-called endocarp: at the bottom of the seed there is a collenchyma sheath, which is also covered by the endocarp. It consists of very strong cells, sometimes thickened until the lumen disappears, which shows a concentric stratification.

The endocarp consists of two elements: first, flattened cells, the cell walls of which are thickened like a net (Fig. 6); the unthickened areas run in one direction and those of the neighboring cells deviate very little from the direction of the former. Furthermore, it consists of spiral vessels that run all the way through the tissue that forms the network-like thickened cells, particularly numerous around the edge of the flat seed. Both elements of the endocarp are lignified. Lignification appears to be more advanced in vessels than in net fiber cells, since after treatment with phloroglucinol + hydrochloric acid or aniline sulfate they show a more intense violet or yellow color than the former.

Ruthenium red (Ruthenium sesquichloride) stains the reticulated cells red. So they contain pectin bodies. Wiesner[20] observed that the endocarp takes on a viscous character when exposed to high levels of humidity and when exposed to liquid water. It is also likely to maintain the liquid, if briefly, of the water supplied to the seed by rain or thaw, thereby helping to keep the seed from drying out. If the fleshy husk of the *Viscum* berries is consumed by birds or is somehow removed, then instead of the entire tissue of the "berry" the endocarp remains the only husk that protects the seeds from harmful external influences.

Treviranus [21] indicates from the thin cuticle that surrounds the seed that it is nothing other than the innermost layer of the fruit substance, which lacks the “viscin”.

Van Tieghem [22] recognized woody elements in the endocarp, but did not discuss the nature of them.

When the fruit is fresh, the endocarp adheres firmly to the seed and cannot be torn off, which happens even when the fruit is dry.

The shape of the seed is very different, depending on whether it includes one, two or three embryos. In the first case it has a flat, elliptical shape and the embryo lies in the direction of the larger axis, the two cotyledons pointing to the bottom and the hypocotyl upwards, which is capitately thickened at the end.

The embryo lies entirely in the endosperm with the exception of the apex of the hypocotyl, which protrudes freely from the endosperm and is covered like the whole seed outwards from the endocarp.

If there are two embryos, the seed is almost in the shape of an equilateral triangle. The hypocotyls are directed towards two corners of the triangle, while the cotyledons, pressed together, turn towards the third corner of the triangle. This corner marks the place where the “seed” is attached to the “berry”. The seed was created here by the growth of two embryo sacs. A separate endosperm has developed in each embryo sac, and so the seed represents two endosperms, which are not completely fused on the upper side and form an indentation at the top. This gives the seed a cordate shape.

In the third case, when there are three embryos, there are two as in the previous case and the third between them is such that its cotyledons are wedged between the cotyledons of the other two seedlings. The axes of all three embryos lie in one plane. In this case, the fruit can take the form of a square.

Van Tieghem states that there are also four embryos, but this has to be seen as a very rare abnormality. According to some authors, the location should have a certain influence on the number of embryos; e.g. Solms-Laubach[23] for the conifer-inhabiting mistletoe forms one embryo, for those living on hardwoods two or more. Kronfeld [24,] on the other hand, also finds a large percentage of lone fruits on maple and poplar. Like Kronfeld, I am of the opinion that the location does not exert this influence on the plant, as I have often found all three cases in the seeds of one and the same branch.

Cross sections through seeds show that the cells of the endosperm are large and parenchymatous. They are full of starch grains and contain acell nucleus and a large amount of chlorophyll grains. This fact highlights Decaisne [25] and says that it is the only example that such a large mass of chlorophyll grains is produced so deep in the tissue.

The cell walls are plentiful with simple pits. They also have intercellular spaces, which are much rarer and narrower at the top than at the inside of the seed. The epidermal cells are convex, outward and very thickened on the side walls (Fig. 1).

They also carry chlorophyll and starch grains like the other cells of the endosperm. If the sections are treated with chlorozinc iodine, the cell walls of the endosperm stain and the outer wall of the peripheral cells differentiate into three layers.

The first from the inside turns purple like the other cells of the endosperm. So it's a cellulose layer. After this comes another, which is two to three times as thick (Fig. 1 c). This turns brown with the reagent mentioned. The outermost one is colored yellow to yellowish brown. After prolonged exposure to the reagent, the middle layer turns deep brown and the outermost layer (the wax layer to be named later) turns brown. The sections, treated with concentrated sulfuric acid, come off completely, with the exception of the middle membrane layer of the epidermal cells. This is colored brown and curls up when the reagent acts on it. Concentrated potash lye colors this layer yellow.

Chromic acid does not dissolve it. With alkane reagents it turns red, just like the outermost layer.

Schulze's mixture makes them stand out more clearly, while the rest becomes more transparent. This middle cell wall layer gives all reactions, like the ordinary cuticle. It is quite powerfully developed and is clearly distinguished from the rest of the cell membrane. This so powerful cuticle does a lot to prevent the water inside the seed from evaporating. It is a known fact that the thickening and cuticularization of the outer walls of the epidermal cells usually keeps pace with the need to protect the underlying tissues against dehydration.

The plants that grow in low-rain climates mostly have such thickening and cuticularization of the outer cell walls of the epidermis, which are able to limit the transpiration of the plants to a very small extent.

The *Viscum* seeds are stuck on branches with viscin and their germination occurs at a time; during which rainfall is sparest, and the air itself is fairly dry. They are therefore only dependent on the water they contain before they ripen. They protect this water from evaporation through the cuticular layer. We will soon see that the tissue mentioned protects the seeds from evaporation in another way. There is another layer over the cuticle, which is even thicker (Fig. 1 w). It is not built so solidly, but it shows many radial cracks. It is colored by chlorozinc iodine, as already mentioned, yellow to yellowish brown. It is almost completely dissolved in ether after prolonged exposure. It partially dissolves in absolute alcohol. After 24 hours of exposure to alcohol, part of it still remains undissolved. It also dissolves in turpentine, carbon disulfide and benzene, as well as in clove oil. After the action of this oil, the differentiation of the cuticular and cellulose layers is particularly evident.

It turns red due to alkane reagents. It is immediately attacked by concentrated sulfuric acid. During the action of the acid, the crevices and cracks in this outermost layer of the wall thickening become larger and the entire layer appears to consist of small rods. These rods then gradually disappear. All reactions that suggest a fat or waxy body.

This fat-like body agrees with that of de Bary [26] and Wiesner [27] as wax, which is deposited on the epidermis of leaves, stems and fruits of many plants. How plentifully the wax coating is deposited on the above-mentioned tissue is evident from the fact that a mistletoe seed lying on a glass plate, with a few drops of alcohol poured over it, leaves a plentiful fat coating on the glass.

The shape found here at *Viscum album* does not match any of the shapes described by de Bary and Wiesner. It is most similar to the rod coating. Some of his rods are completely melted, some of them still have a gap between them. If we look at the wax coating in polarized light, it turns out to be birefringent. For some wax coatings, this behavior was long ago confirmed by Wiesner [28] and this researcher demonstrated that the wax coatings have a crystalline character and are not organized form elements, as de Bary had previously claimed.

I used two methods to roughly determine the melting point of the wax. Some sections made of seeds and stained with alkannin were placed on a slide and placed in a drying cabinet. It was now heated and I checked from time to time by microscopic examination whether melting had already occurred. When the thermometer showed a temperature of 95 ° C, the wax had melted completely.

Small red drops were visible outside and inside the cells. This temperature may have been found a little too high due to the incompleteness of the method. The second method consisted of the following procedure: A seed freed from the endocarp was poured with absolute alcohol on a slide. The wax was partially dissolved and a crystalline residue remained after the alcohol had evaporated. Heating was done by immersing the slide in a beaker filled with hot water, which was adjusted with a thermometer. The wax began to melt at 80° C and was almost completely melted at 85° C. The dry distillation of the wax produces acroles, to prove that this "vegetable wax" also consists or contains fats (glycerides),which was first proven by Wiesner with regard to numerous wax coatings on vegetable skin tissue.[29]

Only now is it clear what facilities the great transpirational protection of the *Viscum* seeds, which also allows them to germinate in the desiccator. The so-called seed is therefore covered with a heavily corked and wax layer that makes it impossible for the water contained in the seed to evaporate rapidly. Only through this facility has the plant been able to survive in the areas of its current distribution area.

If one cuts the seeds so that the hypocotyl is cut across, one sees that the cells of the same are parenchymatous, but smaller than those of the cotyledons, full of chlorophyll grains, which give the whole hypocotyl a dark green color and contain a lot of protoplasm.

The peripheral cells are not isodiametric, but appear to be somewhat radially elongated in cross-section. They are greatly thickened and cuticularized (Fig. 2 c).

This strong cuticularization of the epidermis of the hypocotyl protects it from excessive drying out and of course also from other influences when it emerges from the seed.

A layer of slime cells can be observed around the hypocotyl (FIG. 2 s). Guided by longitudinal sections of seeds one sees that the slime only widens upwards on the ropes where the stem borders on the cotyledons. Its maximum is found where the hypocotyl protrudes from the endosperm (Fig. 3).

There is an indentation on both sides of the stem and the thickened epidermis extends into it for two or three cell lengths (Fig. 3 c). There can be no doubt that this slimy mass has formed from the endosperm. The cells of the endosperm are not at all delimited by this slime, rather you can see how the cells gradually pass into them: the cell walls become thinner and thinner until they are hardly recognizable. The contours of the cells can be seen in the slime itself. Pitra[30] could not decide whether this slime originated from the endosperm cells or whether it was excreted from the hypocotyl.

In the indentation between the hypocotyl and the epidermis of the endosperm there are some slime cells (Fig. 3). These are probably not yet completely repressed cells of the floral axis.

This slime is also at the apex of the hypocotyl. It turns yellow with chlorozinc iodine and red with ruthenium sesquichloride. This slime makes it easier for the hypocotyl to emerge from the endosperm during growth and protects the endosperm from excessive transpiration.

A bundle of vessels consisting of spiral vessels runs through the stem and is divided into two bundles, which go into the cotyledons.

Each embryo has two cotyledons that are so close together that the walls of the touching cells fuse in many places. The tips of the cotyledons are usually free, although the adhesions also occur here.

The greenish white color of the cotyledons catches the eye, while their stem appears dark green and the whole embryo lies in green endosperm.

If two or three seedlings are present in the seed, they usually fuse like the two cotyledons of one and the same seedling, but never so far that the cotyledons represent a homogeneous tissue. Decaisne [31] believed that the cotyledons are overgrown when immature, but free when mature. Treviranus[32] opposed this opinion and claimed that both cotyledons, if there is one seedling, all four, if there are two seedlings, fuse in the fully developed fruit in such a way that no further trace of the previous separation is perceived as a slight framing, inside, however, there is a perfect continuity of the substance.

I cannot confirm everything in this statement either. I have never seen a complete connation of the cotyledons, but I have definitely observed a partial, near the tips, though very rarely. The outer layer of the cotyledons forms an epidermis, the cells of which do not differ in terms of wall thickening from the cells underneath. At the tips of the cotyledons, the cell tissue is particularly delicate and is already noticeable for its lighter, almost white color. The cells of the cotyledons suck up the nutrients from the endosperm.

Viscum orientale .

In order to fully investigate the devices on which the transpiration protection of the seeds of *Viscum album* is based, it was necessary to also examine some seeds of the tropical species which lack this transpiration protection.

The test material consisted partly of alcohol-preserved, partly dried specimens of the type mentioned, which Mr. Hofrath J. Wiesner collected in Java in 1893.

The berries of *Viscum orientale* are very similar in color and size to the fruits of *Loranthus europaeus*. In the fleshy casing, the viscin layer is very poorly developed compared to *Viscum album*. Its elements are identical in shape to those of *Viscum album* and are arranged just as radially against the seed.

The viscin slime turns purple or blue with chlorozinc iodine or with iodine tincture + sulfuric acid and behaves at all against reagents like ordinary cellulose. The outer layer of the fleshy shell is very similar to that of *Viscum album*.

The so-called seed has a cordate shape, like the two embryos from *Viscum album*, but it is relatively thicker and more rounded. It always has an embryo on its side. On the cross sections through the seed one can observe that the epidermal cells of the endosperm are radially stretched and that they have experienced a slight thickening outwards in comparison with that of *Viscum album* (Fig. 4).

In sections treated with chlorozinc iodine, the cell walls turn purple, only the outer layer of the thickened epidermis brown. The latter becomes more concentrated. Not dissolved sulfuric acid, but colored brown. With alkannin it turns red. This layer behaves like the cuticle of an epidermis.

The seed lacks the wax coating described in *V. album* . No trace of the same was found, neither in the alcohol material seeds nor in those taken from the herbarium. The seeds of tropical *Viscum* species do not need transpirational protection because they are in very humid air during the germination and there is also plenty of liquid water available to them.

The epidermal cells are very rich in protoplasm and do not contain starch granules, as I have described for *Viscum album*. The remaining cells of the endosperm are filled with starch granules. The cell walls are provided with simple pits and regularly form intercellular spaces.

Around the stem, the slime on my material had lost its stickiness in alcohol to such an extent that it was no longer able to hold the seedling while cutting, but the seedling fell out immediately.

The stem's epidermal cells are thickened, but relatively weaker than those of *Viscum album*. The elements of the endocarp are parenchymatpis cells and spiral vessels.

The former are neither thickened like a net, nor woody as in *Viscum album,* and they carry many crystal glands of oxalic acid limes.

Viscum articulatum ,

The berries are almost spherical, small and, like those of *Viscum orientale,* have very little viscous slime. It is richer on the flat sides of the seed than anywhere else. The seed is lenticular and has only one embryo that emerges on the side with its stem.

The seed is just as free of wax coating as that of *Viscum orientale*.

In all other anatomical details it is similar to that of *Viscum orientale*.

Summary of results.

1. The viscin slime threads that form when a mistletoe is opened are artificially drawn out cells, which, depending on whether they are pulled out strongly or not, reveal their spiral wall structure or not. They give all reactions of ordinary cellulose and dissolve in copper oxide ammonia.

2. The woody elements of the *V. album* endocarp are: thickened flattened cells and spiral vessels.

3. The slime surrounding the hypocotyl is different from the viscin slime. With chlorozinc iodine it turns yellow, with ruthenium sesquichloride it turns nice red. It forms as a result of slime of the endosperm cells and loses its stickiness in alcohol.

4. The epidermis of the seeds of *Viscum album* is very thickened and cuticular and has a powerfully developed birefringent crystalline wax coating, which often shows radial cracks and crevices. The melting point of the wax is between 80-90° C.

5. As a rule, the cotyledons of two or three embryos do not fuse in such a way that they represent a homogeneous tissue, but their boundary is visible at the growing site.

6. The seeds of the tropical *Viscum* species: *V. orientale* and. *V. articulatum* lacks that wax coating, and even the cuticle is less developed than in *Viscum album*.

7. The endocarp in tropical *Viscum* species differs from that of the *Viscum album* in that the flattened cells are neither thickened in a reticulated shape nor are they woody.

8. The exceptionally strong transpirational protection of the seeds of *Viscum album*, which was first experimentally proven by Wiesner, is accomplished by strong cuticularization of the epidermis of the endosperm and by a powerfully developed wax coating covering this epidermis. The protection against transpiration is further completed by the cuticularization of the epidermis of the hypocotyl and by the slime surrounding the endosperm cells.

Explanation of the pictures.

Fig. 1. Cross section through a seed of *Viscum album* . Magnification 305. w = wax layer with radial cracks, C = cuticle,

Fig. 2. Cross section through the hypocotyl in the endosperm of *Viscum album*. Magnification 305. C cuticle, s = slime around the hypocotyl, e = endosperm cells abutting the slime.

Fig. 3. Longitudinal section through the hypocotyl and endosperm. *Viscum album*. Magnification 140. S = slime around the hypocotyl, C cuticle of the peripheral endosperm cells. *Viscum album*.

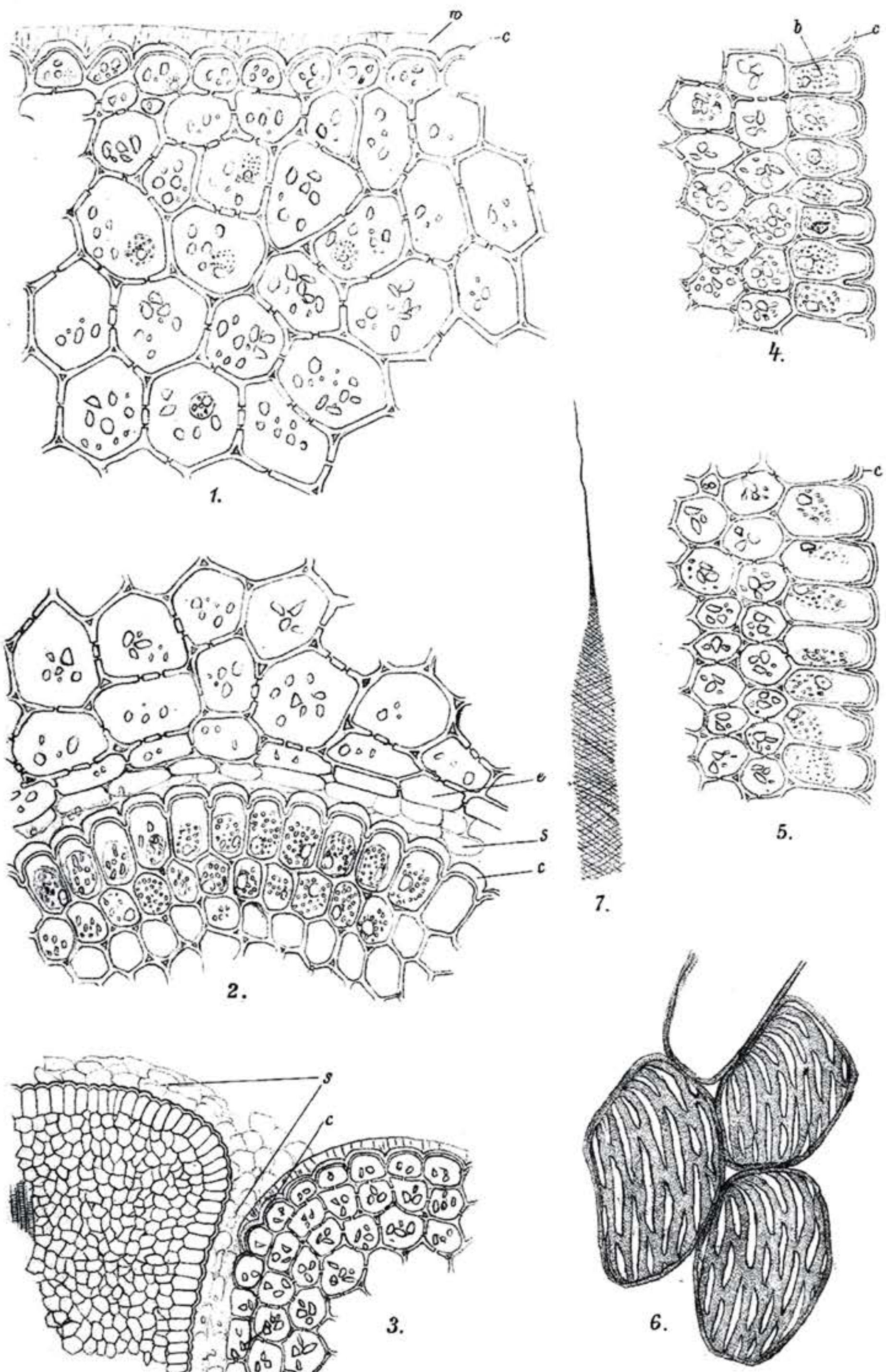
Fig. 4. Cross-section through the peripheral part (endosperm) of the seed of *Viscum orientale* . Magnification 610. C = cuticle, b epidermal cells.

Fig. 5. Cross section through the seed of *Viscum articulatum* . Magnification 610. C = cuticle.

Fig. 6. Network-like thickened cells of the endocarp. Area view of *Viscum album*. Magnification 610.

Fig. 7. A piece of slightly extended *Viscum* cell from *Viscum album* . Magnification 305.

G. Gjokić: Frucht und Samen von Viorum.



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