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Comparative studies on the nature of the fruit slime from *Viscum album* L. and *Loranthus europaeus* L. and their biological significance

by

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In his treatise: “On the anatomy of the fruit and seed of *Viscum*” [1] Gjokić also presented the results of his examination of the fruit slime from *Viscum album*. Professor Dr. Hofrat J. Wiesner entrusted me with the task of examining the slime of the fruit of *Loranthus europaeus*. I did the comparative work by repeating and reviewing the investigations made by Gjokić, but doing the same and others with *Loranthus* slime. This made it possible for me to be able to recognize even minor differences with certainty.

Before I start to present the results of my investigation, however, as a guide I would like to give the views of some researchers about slime.

Based on the reaction to iodine and chlorozinc iodine, Tschirch [2] diffferentiates between cellulose mucilages, which give the known cellulose reactions, real slimes [mucilages?] and gums, which turn more or less yellow to brown with chlorozinc iodine, and finally amyloid (for example in the cotyledons of the seeds of *Tamarindus*). This is already bleached by iodine alone. Real slime also differs from cellulose slime in that the former also gives mucic acid in addition to oxalic acid when oxidized with nitric acid, while the latter only give oxalic acid in this process. As for solubility in copper oxide ammonia, Tschirch notes that the latter two types of slime are insoluble in them. As an exception, he mentions the sand plantain slime (from *Plantago Psyllium*).

Czapek [3] says: “From a chemical point of view, the slimes are still very poorly known. Relationships with pectin substances and rubber may exist, but have never been proven with certainty. All slimes form colloidal solutions in water.”

A classification, also accepted by Strasburger [4], comes from Mangin [5]. This divides the slime into cellulose, pectose and callose mucilages. The pectose slime pretty much corresponds to Tschirch’s real slime. Mangin also differentiates between mixed and undetermined slimes.

Gitaud [6] also gave a classification of the slimes, although this is unnecessary for our purposes.

I now go to the treatment of the actual test substance. Above all, I want to give Gjokić’s [7] information that coincides with my findings.

The fruit of *Viscum album* has the appearance of a berry of a fairly pure white color. One can differentiate between them: a coarse outer skin, a completely mucilaginous layer, the viscin layer that interests us, finally the 1 to 3 embryo-containing kernel [core, pip], the so-called seed, which is surrounded by slime all around.

If you crush a berry between your fingers, the kernel swells out, surrounded by a layer of slime. A viscous coating also remains on the outer skin.

If we examine each of the two layers individually under the microscope, we see that they do not have the same appearance. The outer layer, which hangs on the outer skin, consists almost entirely of threads, while the inner layer presents itself to the armed eye as a mixture of the threads mentioned, with a large number of clumps clumped into balls. Gjokić seems to have only examined the outer layer, as his information indicates only with this match my findings.

According to Gjokić and Czapek [8], each thread of this layer corresponds to a cell which, in the form of a spindle, was pulled apart at its pointed ends. You can easily see the cellular body, especially after tinting with methylene blue, since the plasma has turned lively blue.

If we now put a little slime of the outer layer of the slide without tearing it apart, and let chlorozinc iodine be added, the slime slowly stains and only on the edges. Pulled apart, it stains quickly, and the threads become purple, the cells yellow to light brown. This is not in accordance with Strasburger [9], according to which iodine compounds hardly affect cellulose slime.

In addition to Gjokić's previous statement, I found the following of his statements confirmed.

With iodine tincture and sulfuric acid, the slime threads turn blue, the cells yellow to brown.

Ruthenium red, a dye recommended by Mangin [10], in 0.02% aqueous solution, colors the slime slightly pink, just as cotton or sulfite cellulose is colored very little pink.

Congo red causes a very lively red color, which hardly changes even after careful washing.

The blue color that shows on the slime after prolonged exposure to methylene blue is completely eliminated by continuous washing with water, so that the threads remain colorless. In contrast, the cell content stores the dye in itself.

The slime does not dissolve in water, or only very little. When I filtered such water containing slime that added alcohol (96%) to the clear filtrate, a slight turbidity was evident, the examination of the precipitation was impossible.

The slime is completely soluble in copper oxide ammonia. Later we will also get to know some slimes, which are soluble in this medium, so that Tschirch's[11] statement that real and cellulose slimes are insoluble, but as a rule with a few exceptions. The slime also dissolves in sulfuric acid.

As far as the information Gjokić agree with my findings. He ignores the fact that the slime threads are double refractive because they glow iridescent in the dark face of the crossed polarizer. But some of his results are in direct contradiction with mine.

So he writes that korallin soda stains the slime. I was able to remove the dye by washing it out, so that there was hardly any trace of a tincture.

He also states that the slime does not dissolve in potash lye, but only swells. This is certainly not the case. I digested slime with strong potash lye for 3 to 4 days, filtered and added alcohol drop by drop. A flaky precipitate separated out at the point of contact. With more alcohol added and subsequent shaking, the flakes clumped together into lumps. This precipitate gave all specific cellulose reactions just like the original slime. If you crushed a lump of the precipitate on the slide, you could see under the microscope that it had broken up into very thin threads. These also proved to be double refractive.

The solubility in potassium hydroxide is not only a property of the cellulose slime, but also of the pure cellulose. Tollens [12] writes that 10% sodium hydroxide solution, which has the same effect as potassium hydroxide, dissolves up to 40% of cellulose digested with it and that alcohol from the solution precipitates an amorphous mass (according to Koch) [13]. B. Quadrat[14] writes similarly. My experiments with sulfite cellulose and salt slime, which is also a cellulose slime, also confirmed this information.

The slime of the outer layer of the *Viscum* fruit shows all the reactions that are typical of cellulose, so it belongs to the cellulose slime. However, the lumps of the inner layer behave differently. Nowhere did I find an indication of the presence of this layer of slime.

With chlorozinc iodine or with iodine tincture and sulfuric acid, these lumps turn yellow to brown, while with methylene blue they turn intensely blue. They differ significantly from the slime threads and belong to Tschirch's real slime to be discussed later or Mangin's pectose slime.

The lumps are soluble in copper oxide ammonia, potassium hydroxide solution and in concentrated oxalic acid solution. The precipitate that is precipitated with alcohol gives the same reactions as the slime lumps, and it also proves to be optically inactive.

I also noticed a few droplets of fat suspended in the slime. I do not dare to decide whether they originally belong to the epicuticular wax layer of the "seed" found by Gjokić or the mucous layer. I recognized that it was a fat or waxy body from the stains with Sudan III, alkane tincture and osmic acid.

The construction of the fruits of *Loranthus europaeus* is largely the same as the construction of the fruits of *Viscum*. The color of the fruit is a rather lively yellow

If you examine the pale yellow slime under your microscope, you see a homogeneous mass, in which highly refractive droplets are embedded. There is no trace of cells. The whole slime layer is the same.

If we now tinge with chlorozinc iodine or tincture of iodine and sulfuric acid, we get a yellow to brown color.

Methylene blue colors very strongly and lively blue. Congo red also stains very well and lasts. This behavior of the dye agrees with the information provided by Heinricher [15] and Chalon [16], according to which Congo red, which is otherwise a typical absorbent for cellulose, also tingles with pectin substances, callose, lignified and corky cell walls.

Safranin also colors. Korallin soda can be completely removed again. In contrast, aniline blue stains very strongly without discoloring during washing. All these stains remain in 1 to 2% boric acid for some time, but quickly disappear in acids, glycerin and even in alcohol.

Ruthenium red gives a strong and extremely durable tint that can hardly be reduced by washing for days.

The slime of *Loranthus* coagulates in lead acetate and is optically inactive.

It swells quickly in water, breaks up into flakes and dissolves a little; if you add alcohol to a clear solution, a weak precipitate will fall out. It also dissolves in copper oxide ammonia, potassium hydroxide solution and oxalic acid. The behavior towards the former solvent is not special since, according to Frank [17], *Plantago* slime is also soluble in it; however, flaxseed slime is insoluble in it. And yet both are pectose slimes. Perhaps two modifications can be assumed among the pectose slime, similar to what Husemann [18] cites for the slime from *Cydonia* .

The solubility of the fruit mucilage from *Loranthus* in potash lye and oxalic acid is congruent with the information given by Wiesner [19], according to which pectin substances are soluble in these agents. However, the oxalic acid must be concentrated. Alcohol precipitates out of the solutions, showing all the reactions and properties of the slime.

The reactions shown show that the fruit slime of *Loranthus europaeus* is a pectose slime.

The previously highly refractive droplets in the slime turn deep red-brown with iodine compounds, peculiarly yellow-red with Sudan III, pink-red with Alizan tincture, gray to black-gray with osmic acid. They dissolve completely in benzene and carbon disulphide. So they are drops of a fat or waxy substance.

There seem to be relationships between the individual slimes and the rubber substances. Because, according to Kirchner [20], the various slimes disintegrate when cooked with 1-25% acid in cellulose and gum, which the latter turns into sugar when cooked again. Only the type of decay and the quantities of the decay products differ with different slimes, “So the plant slime is a glycoside or ether-like body made of cellulose and rubber.” (Kirchner.)

The previously mentioned behavior of gum against nitric acid is similar to that of Tschirch's real slimes.

So the results are: the slime of

	Viscum changes color		*Loranthus changes color*
with :	outer layer	inner layer	
Chlorozinc iodine	violet	yellow to brown	yellow to brown
Iodine ± sulfuric acid	blue	yellow	yellow
Methylene blue	blue	blue	blue
Congo red	red	red	red
Ruthenium red	faint pink	pink	pink
Korallin soda	no	no	no
Safranin	no	red	red

Regarding solubility:

	Viscum		*Loranthus*
in:	outer layer	inner layer	
Copper oxide ammonium	soluble	soluble	soluble
Potash lye	soluble	soluble	soluble
Conc. oxalic acid	insoluble	soluble	soluble
Water	undetectable	weak	weak

So the slime is: cellulose, pectose, pectose slime. (Addendum: Giordan [21] recently discovered South American Loranthaceae with rubber up to 20% in place of the slime, which corresponds to the viscin layer of *Viscum*.)

As we have seen, the slime layer of the fruit of *Viscum* consists of two different types of slime. To explain this fact, two aspects seemed worth considering: 1. Which task does the slime have in the germination process? 2. Does it have a dissemination role?

Wiesner [22] refuted Quérin’s [23] statement that the *Viscum album* slime was indispensable for germination. Wiesner showed that the slime is not only not necessary, but also has an almost germination inhibiting effect, which explains the strange germination delay of the seeds of *Viscum album*. He attributes this inhibitory effect to substances that develop in fresh slime and prevent germination, but in the spring disappear from the slime. Various experiments, which I made with different slime, suggest that in addition the lack of oxygen caused by the slime is one of the causes of the inhibition of germination.

For the experiments I used the slime of *Cydonia*, *Plantago*, *Lepidium sativum*, *Viscum* and Salep. I let cress seeds germinate freely on damp paper, on slime and embedded in slime under otherwise identical conditions. In the first case they germinated after 30 to 36 hours, in the second case in 3 to 5 days, but in the third case in the slime full of *Plantago Psyllium* not even after 12 days, in that of *Lepidium* not after 10 days. Germination was completely prevented by the slime. On the other hand, I could not notice any delay when using a thin gelatin of *Cydonia* slime or Salep, which would be the case if soluble, non-inhibitory substances were contained in the slime in large quantities. Here, the lack of oxygen caused by the slime is at least one cause of the inhibition of germination.

My diffusion experiments show that for air, slime is not at all or only very slightly permeable. Water was sucked 80 to 85 cm into an approximately 1 *m* long glass tube with an internal width of 5 to 6 *mm* and sealed airtight at the upper end with slime. Despite the rather strong pressure on the slime, the height of the water column remained constant until the slime had dried up. This lasted 14 days for *Plantago* slime, 18 days for Salep, 16 days for viscin slime etc.

Small vials were filled with indigo vat and then, as above, sealed with slime. The vat, which was in a bottle without a slimy seal, lost its beautiful wine-yellow color in 2 to 3 days. In the sealed vials only after the slime has dried up. So with closure with *Plantago* slime after 12, with viscin after 14 days.

Green crystals of ferrous sulfate were embedded in slime on a watch glass, while others were exposed to the free influence of the air. While the latter had already turned completely brown after 4 to 5 days, those embedded in *Plantago* slime remained completely green after 10 days. I achieved similar results with the other slimes.

These experiments show with certainty that the slime is at least extremely difficult and slowly diffusible for air, and therefore also for oxygen, so that the assumption that lack of oxygen is a cause of the inhibition of germination has some validity.

However, if one considers the lack of oxygen as the cause of the inhibition of germination, it is not easy to explain the fact that in completely intact fruits the hypocotyls emerge in the spring (April-May), despite being completely surrounded by slime (Kronfeld [24]).

Wiesner [25] says that the slime loses its stickiness towards spring and the germination inhibiting substances disappear. Perhaps the decrease in stickiness makes oxygen access easier, so that the process mentioned can be explained in this way.

The fact that tropical Loranthaceae do not go through a period of rest, even though they also have seeds covered by a layer of slime, albeit a small one, can be explained by the lack of inhibitors or by the hygrophyllic character of these plants, in which the slime is washed away by heavy downpours. *Viscum album,* however, is a xerophyte in the germination stage (Wiesner [26]). The assumption that the slime from *Viscum album* serves as a water reservoir is invalidated by L. Linsbauer's studies [4], according to which the viscin slime is only slightly hygroscopic.

The rest period, which can be reduced to 1/6 under the most favorable conditions (Wiesner [27]), presents itself to us as an inherited trait acquired through adaptation (Wiesner).

As a far more important reason for the presence of the two types of slime in the fruit of *Viscum,* I believe that the adaptation of the berries to the distribution by birds can be considered. It is an adaptation if *Viscum*, whose white berries would not be visible in the snow in winter, is evergreen, but *Loranthus* with its yellow fruits throws its leaves off in autumn.

Kerner [28] and Kronfeld [29] say that glue mistletoe is widely distributed only by birds. The latter, however, shows that by dropping the berries from branch to branch, they can stick on, which he confirmed by experiments. However, this type of distribution is only possible on the trees on which the mother plant lives, or at most in a small neighboring area. However, various birds ensure the further spread. They sow the seeds by eating the slime, but stripping off the kernel from the branches, or they consume the whole fruit and throw out the kernel with the cast [pellet] or faeces. Now the arrangement of the fruit, that the outer layer of slime consists primarily of cellulose slime, the inner layer of pectose slime, appears to be extremely useful. Because cellulose is digestible. Neumeister [30] says that although it is not changed by the digestive secretions, it is at least partially solved by bacterial influences. In herbivores, a significant fraction of the fed cellulose is undetectable in the faeces. Hofmeister's [31] experiments with un-disinfected horse intestinal fluid showed that up to 78% of cellulose is dissolved by it. Volt [32] indicates that the same thing happens with cellulose slime. Thereafter, when dogs are fed Salep, the slime is largely absorbed. The same cannot be proven in the excrement.

Pectin substances behave differently. These are very difficult to digest. Neumeister [33] teaches that pentaglucoses or pentoses or their parent substances, the so-called pentosans (pectin substances, vegetable gum) are only very imperfectly absorbed by organisms. Wiesner [34] also says that pentaglucoses are difficult to decompose and pass unchanged into the urine.

The fact that the inner layer of slime consists of pectose slime will probably have the purpose of preserving the slime coating necessary for the seed as protection against germination due to its indigestibility, while the cellulose layer , although it also has an antiseptic effect, serves as food for the birds. If this protection against germination were not available, the seeds could start to germinate at the beginning of January, since the heat necessary for germination (+8 to 10° C.) is sometimes available at this time. A subsequent frost would then destroy the whole generation (Wiesner).

For birds that remove the slime and strip the kernels with their beaks, the layer of pectose slime probably also prevents the seed from being damaged by the beak. Because, if only cellulose slime were present, the bird would also injure the seed when the slime was removed.

Other birds consume the seed along with the slime. The kernel itself, however, is ejected with the undigested pectose slime either with the cast or the faeces. However, due to the indigestibility of the pectose layer, a layer of slime around the kernel is preserved.

Here there is an adaptation of the fruit to the spread by birds, similar to *Taxus baccata*, for example, whose fruits are also adapted to the spread by birds, since the red aril is not poisonous and edible, while the seed is inedible due to its toxicity.

Since the layer of slime in *Loranthus* consists only of pectose slime that is difficult to digest, the abundant fat droplets will probably be used to feed the birds, since the *Loranthus* berries also depend on birds for their spread.

If we now look at the results obtained, the following are revealed:

1. The slime complex of the fruit of *Viscum album* consists of two different types of slime: a cellulose slime layer, which mainly forms the outer layer, and a pectose layer, which forms the inner slime layer. Droplets of a fat or waxy substance are present in small amounts.
2. The slime of *Loranthus europaeus* is similar and consists only of pectose slime in which fat droplets are suspended in bulk.
3. Both types of slime have an germination inhibitory effect. The cause of this are germination inhibiting substances (Wiesner) and probably also the exclusion of respiration due to the impenetrability of the slime to oxygen, which, however, forms a requirement for germination.

4. The division of the *Viscum* slime into an outer, digestible cellulose layer and an inner, indigestible pectose layer appears as an adaptation of the fruit to the spread by birds.
5. In the fruit of *Loranthus,* the numerous droplets of fat are likely to serve as food for the birds that spread the fruit, since the slime is difficult to absorb as pectose slime.

I would like now to express my sincere thanks to Professor Dr. Hofrat J. Wiesner for his extremely benevolent and work-based support and promotion of the work at hand.

Footnotes

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