Mit den beiden Methoden, deren Leistungsfähigkeit bekannt war, *ist kein Piezoeffekt nachweisbar.* Die Versuche von HETTICH und STEINMETZ[1] und MASON und OWSTON[2] geben dasselbe Ergebnis. Dagegen haben sich die Resultate von ROSSMANN[3] nicht bestätigt, wiewohl der von ihm behaupteten Existenz von elektrischen Zwillingen Aufmerksamkeit geschenkt worden war und teils seine eigene Versuchsführung[4] reproduziert wurde. Aus ROSSMANNS zahlenmässiger Angabe für den Piezomodul (Effekt 10mal grösser als Turmalin in der Methode von BERGMANN) würde sich eine elektromechanische Kopplung vom unwahrscheinlich hohen Wert 0,1 ergeben. Aus diesem Ergebnis darf noch nicht ohne weiteres gefolgert werden, dass Eis eine nichtpolare Struktur hätte, indem nämlich eine äusserst kleine Kopplung auf Grund der sehr lockeren Struktur des Eises möglich ist.

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Eidg. Institut für Schnee- und Lawinenforschung, Weissfluhjoch-Davos, den 10. Februar 1953.

Summary

Piezoelectricity of ice was investigated by two methods whose sensitivity was determined separately. No effect has been found. An electromechanical coupling of 10^{-3} or a corresponding piezoelectric modulus of $1{\cdot}5 \times 10^{-9}$ (stat. clbs. dyn^{-1}) would have been the lower limit for detecting piezoelectricity. Special attention was given to the selection of homogeneous crystals for these tests.

[1] A. HETTICH und H. STEINMETZ, Z. Phys. *76*, 700 (1932).
[2] B. J. MASON und P. G. OWSTON, Phil. Mag. *43*, 1911 (1952).
[3] F. ROSSMANN, Exper. *6*, 182 (1950).
[4] Mitteilung von F. ROSSMANN und F. JONA, Phys. Inst. ETH., Zürich (April 1952).

Chemical Components of Plant Cuticle, with Special Reference to the Triterpenoid Constituents

There has been no report on the chemical components of plant cuticle since the work of LEE and PRIESTLEY[1] and of LEGG and WHEELER[2]. Further, the true character of cutin has scarcely been studied. We have examined microchemically the leaves of some 600 kinds of Japanese plants and reached the conclusion that nearly 300 contain triterpenoids such as ursolic acid, etc.

The microchemical procedure is as follows: *Procedure I.* A drop of acetic anhydride is placed on a plant leaf, which is heated from below by a small flame for few seconds until the substance contained in the cuticle of a leaf has dissolved in the liquid. It is then sucked into a capillary tube of *ca.* 1 mm diameter. To this is added a very small quantity of conc. sulphuric acid, to which the red coloration of LIEBERMANN's reaction occurs if some triterpenoids exist in the leaf.

Procedure II. Add a crystal of trichloro-acetic acid to the microsublimate of plant leaf in a small test tube, and heat up to 110°C, a violet or red colour occurs in general, while in the case of phytosterols, cholesterol or related substances the discoloration begins between room temperature and 60°C. By this means, the triterpenoid substances detected by the procedure I. can be proved to be triterpenoids.

Amongst the plants examined, the following families were found to have a high content of triterpenoids: *Caprifoliaceae, Scrophulariaceae, Apocynaceae, Gentianaceae, Oleaceae, Piloraceae, Ericaceae, Araliaceae, Cornaceae, Myrtaceae, Eleagnaceae, Thymelaeaceae, Theaceae, Ulmae, Aquifoliaceae, Rosaceae, Trochodendraceae, Betulaceae, Myricaceae.* We also found that melissyl alcohol is widely distributed, accompanying the triterpenoids. Since the cuticle of the leaves of *Ilex latifolia* R. Br. was the thickest among the hundreds of plants examined, these leaves were boiled with 60% zinc chloride solution acidified with hydrochloric acid and washed with water. Finally, the cuticle was peeled off. We obtained the following analytical data of the cuticle:

Part soluble in methanol and carbon tetrachloride	29·0 %
Part saponifiable by 5% methanolic caustic potash	24·6 %
Cellulose, uronides, lignin, etc.	60·4 %
Ash	6·0 %
Total	100 %

The results of analysis in detail are:

Ursolic acid	0·68%
Melissyl alcohol	0·32%
Lignin[1]	38·0 %
Cellulose	10·2 %
Resene	10·2 %
Fatty acid	12·0 %
Polymer of fatty acid[2]	11·0 %
Uronide[3]	2·0 %
Glycerol[4]	trace

The similarity of the results to those obtained with the cork of *Quercus Suber*[5] is noteworthy.

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Zusammenfassung

Die Tatsache, dass Triterpenoid ein allgemeiner und wichtiger Bestandteil der pflanzlichen Kutikula sein muss, wurde nachgewiesen.

[1] Only 1·5% of methoxy group could be estimated.
[2] On acidifying the saponified liquid, there separated at once a solid, insoluble in any solvent, which became a black hard mass upon drying.
[3] Estimated by LEFÈVRE's method. In addition glucose and the derived glucosazone were found to exist by paper chromatography, using the solvent, Collidine: Phenol: Acetic acid: Water = 4:2:3:1.
[4] Detected by the method of F. FEIGL: « Qualititative Analyse mit Hilfe von Tüpfelreaktion».
[5] H. E. FIERZ-DAVID, Exper. *1*, 160 (1945). – DRAKE, J. Amer. Chem. Soc. *57*, 1570 (1935).

Swollen Starch Grains and Osmotic Cells

Surveying the status of starch chemistry, MEYER[1] simply states that "the swollen starch grains behave like little osmotic cells which shrink in a hypertonic

[1] B. LEE and J. PRIESTLEY, Anal. Bot. *38*, 525 (1924); *39*, 755 (1925).
[2] V. LEGG and R. WHEELER, J. Chem. Soc. *1929*, 2444.

[1] K. H. MEYER and G. C. GIBBONS, Adv. Enzymol. *12*, 341 (1951). – K. H. MEYER, Exper. *8*, 405 (1952).

medium". FREY-WYSSLING[1] also concludes that the wall of the sac must be semipermeable, because "in hypertonic solutions the sacs contract and in hypotonic solutions they swell". The semipermeability would be caused by a submicroscopic reticular structure of the amylopectin. This structure, however, could not be demonstrated unambiguously in pictures taken with the electronic microscope.

Years ago osmotic processes were also held responsible for the development of the sac during the swelling of the starch grain, until it was demonstrated by the author[2] that the formation of the sac is exclusively due to a process of tangential swelling, as confirmed later by BEAR and SAMSA[3]. There are a number of considerations which *a priori* make it unlikely that the starch membranes are semipermeable.

First of all it may be remarked that the above mentioned generalized statements refer to potato starch grains only, where usually no rupture of the walls takes place during swelling. For cereal starch grains e.g. which behave in a different way[4] it would be difficult from the first to assume the existence of semipermeability.

Secondly, we know that the walls of swelling potato starch grains (to which the present discussion further will be limited) become permeable to amylose, as the linear fraction diffuses out during the later stages of swelling. Therefore semipermeability of the walls can only be a transient feature which gradually disappears.

Since different starch grains do not swell at the same time under the same conditions, the bigger ones always first, they should show different stages of semipermeability. Generally it would be impossible to select the sacs showing the correct stage for study.

On top of this it is easy to demonstrate that such stages of semipermeability can not exist for the starch grain as a whole. Each layer of the granule of necessity has a greater swelling power than the layer it surrounds and, therefore, will extend the latter while swelling. As a result the layers situated near the hilum will be most extended and their structure will break down at an earlier stage than that of the more peripheral layers. The layers of a starch grain do not show fundamental differences in structure[5]. Therefore at each stage of swelling a gradual transition of structurally more disturbed layers to less disturbed layers will exist from the inside towards the outside. This is one of the reasons why in the beginning amylose accumulates inside the cavity formed.

Now it will be clear that a possible stage of semipermeability may occur in perhaps only one or two layers at a time. Each layer will then pass through that stage, first of all the most central layer, then the layer surrounding it, and so on. Only when the most peripheral layer would pass the stage of semipermeability could we compare the swollen starch grain to an osmotic cell. This stage will be of short duration and it is highly improbable that we would be able to select a sac which exactly meets the requirements.

Lastly, the general statement about the action of hypertonic solutions could refer only to substances which do not cause swelling by breaking hydrogen bonds between adjacent starch molecules, but of those substances there are not many. Amongst the electrolytes concentrated solutions of salts like sulphates and chlorides, and also a saturated solution of NaOH or H_3PO_4 will not cause swelling of starch grains[1]. Sugars are classic examples of organic substances inhibiting swelling.

Therefore, considerations based on our knowledge of the swelling of a starch grain lead us to the conclusion that we cannot expect the sacs to behave like osmotic cells. Experiments confirm this conclusion.

Potato starch grains were heated in a 33% glucose solution at a temperature of 90°C, when they formed well developed persistent sacs[2]. Swelling is limited under these conditions due to the limited amount of water available. After repeated washing and centrifuging in distilled water no trace of glucose could be found inside the sacs.

Before it is concluded that the walls of the sacs are permeable to glucose, we have to prove that the sugar had entered the sacs before they were washed out, as we know[3] that native starch does not absorb sugar from a watery solution. However, the original presence of glucose inside the sacs is easily demonstrated by adding FEHLING's solution to the hot suspension.

As the author had no knowledge of actual measurements performed on sacs in different solutions, a number of such measurements were done with the aid of a DE FONBRUNE micromanipulator and using phase contrast. One difficulty in this type of experiment is the plastic deformation sacs may undergo during transport from one droplet to another. The oil chamber of DE FONBRUNE however considerably decreased this danger, as it was easy to have isolated sacs always surrounded by a sufficient quantity of water. Also different orientations may simulate contraction or expansion.

Sacs of different sizes, and also fragments of the walls obtained with the aid of a Waring Blendor, were isolated and transported into concentrated solutions of $MgCl_2$, NaCl, KCl, $(NH_4)_2SO_4$, $Th(NO_3)_4$ and NaOH, which did not cause swelling of the native starch grains. In all cases no contraction, but always a slight swelling was found. The fact that a very small addition of NaCl gives a sharp drop in viscosity[4] will have to be explained in another way.

Contraction however took place in glucose solutions. When the sacs were brought back into water they resumed the original shape and dimensions, and this process could be repeated ad libitum, several changes being possible within a few seconds.

It is important that the same contraction is demonstrated by fragments, e.g. the two halves of a sac which had been cut lengthwise, or Waring Blendor fragments. Therefore contraction of the sacs in glucose solutions is not due to osmotic behaviour.

Moreover an originally smooth sac may show rumples after contraction. These rumples do not disappear after swelling in water, as we would expect when osmotic pressure was involved. Once a sac shows a local deformation this is maintained. The rumpled sacs still maintain their full elasticity.

It is a well-known fact that swollen starch grains can be stained with congo red. It was found that the stain remains absorbed even after strong contraction in concentrated glucose solutions. This indicates that the sugar molecules replace free water molecules and that contraction is caused by a closer association of the starch

[1] A. FREY-WYSSLING, Exper. *8*, 101 (1952).
[2] N. P. BADENHUIZEN, Rec. Trav. Bot. néerl. *35*, 559 (1938).
[3] R. S. BEAR and E. G. SAMSA, Ind. Eng. Chem. *35*, 721 (1943).
[4] N. P. BADENHUIZEN, Baker's Digest *25*, 1 (1951).
[5] N. P. BADENHUIZEN, Transact. Faraday Soc. [B] *42* 255 (1946).

[1] N. P. BADENHUIZEN, *De Chemie en de Biologie van het zetmeel* (Oosthoek, Utrecht, 1949), p. 221.
[2] R. T. WHITTENBERGER and G. C. NUTTING, Ind. Eng. Chem. *40*, 1407 (1948).
[3] A. LEULIER and A. COEUR, J. Pharm. Chim. [8] *27*, 241 (1938).
[4] K. H. MEYER, Adv. Coll. Sci. *1*, 143 (1942).

molecules. The elasticity of the contracted sacs further points to the presence of watermantles round the liberated OH-groups, a condition which evidently is necessary for the absorption of congo red.

Finally, sediment volumes were measured in a number of solutions of different substances. 3 g starch was stirred with 72 ml water and the suspension heated on a waterbath until a temperature of 63°C was reached. After centrifuging the sediment volume was 40 ml. The sediment (containing about 93% of water) was mixed with 120 ml of water, 38% glucose solution or saturated NaCl solution. After 24 h the sediment volume was measured and expressed in percentage of total volume. The same value (35%) was found in all cases, so that eventual contractions could not be demonstrated by this method.

The de Fonbrune micromanipulator used in the experiments was generously placed at our disposal by a grant from the South African Council for Scientific and Industrial Research.

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Zusammenfassung

Es wird gezeigt, dass die Wände gequollener Stärkekörner nicht semipermeabel sind und dass demzufolge die Blasen nicht mit osmotischen Zellen verglichen werden können. Quellung und Schrumpfung der Blasen unter dem Einfluss verschiedener Substanzen sind die Folge kolloid-chemischer Strukturänderungen in der Blasenwand selber.

Untersuchungen über chemische Zusammensetzung und Aufbau des Hirschgeweihes

Über den Aufbau und die Gestaltung des Hirschgeweihes wurden zahlreiche interessante Beobachtungen mitgeteilt, so dass Wachstumsbeginn, Ausdehnung, Abwurf usw. gut bekannt sind[1]. Durch die Arbeiten von WISLOCKI und Mitarbeitern[2] sind wir auch in anatomisch-histologischer Hinsicht über den Geweihaufbau orientiert. Hormonale Beeinflussungen desselben konnten zum Beispiel durch Verabreichung von Thyroxin gezeigt werden, welches das Geweihwachstum anregt[3].

[1] H. BRUHIN, Physiol. comp. oecol. (im Druck).

[2] G. B. WISLOCKI, Amer. J. Anat. *71*, 371 (1942). – G. B. WISLOCKI, H. L. WEATHERFORD und M. SINGER, Anatom. Rec. *99*, 265 (1947). – C. M. WALDO, G. B. WISLOCKI und D. W. FAWCETT, Anatom. Rec. *84*, 27 (1949).

[3] N. G. LEBEDINSKY, Acta biol. Latvig. (1939).

Um die chemische Zusammensetzung des konsolidierten Geweihes mit derjenigen der grossen Röhrenknochen vergleichen zu können, haben wir einige Untersuchungen durchgeführt, deren Resultate aus der Tabelle I hervorgehen.

Der Quotient Ca/P beträgt somit 1,63 bis 1,75, im Mittel 1,64, und entspricht dem theoretischen Wert (1,665) für Hydroxylapatit $3Ca_3(PO_4)_2 \cdot Ca(OH)_2$. Es besteht kein Zweifel, dass konsolidiertes Geweih und Knochen chemisch weitgehend identisch aufgebaut sind und die anatomisch-histologischen Befunde, welche darauf hinweisen, von dieser Seite her eine Bestätigung erfuhren. Unsere Ergebnisse über die Zusammensetzung der beiden untersuchten Geweihe stimmen mit einer von WEISKE[1] publizierten Analyse eines Hirschgeweihes überein, welche gleichfalls einen Ca/P-Quotienten von 1,66 ergab.

Wir untersuchten ferner den sogenannten Bast, der bekanntlich als lebende, behaarte Haut das Hirschgeweih während der Dauer seines Wachstums umgibt und später eintrocknet, abstirbt und abgefegt wird. Die Resultate der chemischen Prüfung zweier lufttrockener Proben sind aus der Tabelle II ersichtlich:

Tabelle II
Analyse von Bastproben

Probe	Feuchtigkeitsgehalt %	in % des Trockengewichtes			
		Asche	Gesamt-N	P	Ca
A	49,0	3,14	11,73	0,21	0,25
B	49,8	3,04	11,76	0,25	0,17

Die Werte zeigen, mit Ausnahme derjenigen für das Kalzium, eine gute Übereinstimmung.

Wenn das Skelettwachstum schon längst beendet ist, erreicht im späteren Alter das Geweih seine grösste Ausdehnung. Wie wir zeigen konnten, ist der in 3–4 Monaten erfolgende Aufbau mit einer intensiven Beteiligung am Phosphatstoffwechsel begleitet. Wir haben einem zweijährigen Damhirsch (geboren am 15. 8. 1949) mit im Wachstum befindlichem Geweih am 27. 7. 1951 Phosphat, enthaltend radioaktiven Phosphor, injiziert und das Tier nach 13½ h getötet[2]. Ein zweiter einjähriger

[1] H. WEISKE, Landwirtschaftliche Versuchsstation *20*, 35 [siehe auch Tabulae Biologicae III, S. 371 u. 381 (1926)].

[2] K. BERNHARD und G. BRUBACHER, Z. physiol. Chem. *290*, 237 (1952).

Tabelle I
Analyse von Skeletteilen und Geweihproben eines Damhirsches

	Feuchtigkeitsgehalt %	Asche		Ca-Gehalt der Asche %	P-Gehalt der Asche %	Quotient Ca/P
		in % des Trockengewichtes	in % des Frischgewichtes			
Tibia	13,3	54,6	63,0	40,6	17,9	1,75
Metatarsus	18,2	49,4	60,3	40,9	18,7	1,69
Femur	28,4	42,5	59,4	40,6	19,3	1,63
Metacarpus	23,9	44,8	58,8	40,3	18,4	1,69
Ulna	18,6	46,6	57,3	40,3	18,9	1,65
Humerus	24,2	39,3	51,8	39,0	18,4	1,64
Geweih 1	7,8	46,4	50,3	40,3	19,5	1,60
Geweih 2	6,2	47,3	50,4	40,6	18,9	1,66