Ainsi, le pH normal du liquide de la chambre antérieure, mesuré à l'aide d'un électrode de verre, s'élève en moyenne à 7,59 (1er groupe de 26 cas) et à 7,57 (2e groupe de 10 cas). Par une méthode colorimétrique, KINSEY a obtenu la moyenne de 7,60 (valeurs extrêmes: 7,43–7,81)[1].

Trois heures après l'injection de Diamox, nous observons une baisse significative du pH de l'humeur aqueuse. Cet effet n'est pas plus marqué, dans le second groupe traité par 200 mg/kg, que chez les autres animaux ayant reçu 100 mg/kg du médicament.

Dans l'interprétation de ces résultats, il n'est pas possible d'exclure complètement la participation de l'acidose sanguine due à l'action rénale de l'acétazolamide. Cependant, on peut admettre que la baisse du pH de l'humeur aqueuse reflète, pour une part du moins, une inhibition spécifique de la carboanhydrase du corps ciliaire. Cet effet est associé vraisemblablement à une diminution du taux des bicarbonates; des expériences sont en cours à ce sujet.

La chute du pH de l'humeur aqueuse, sous l'effet du Diamox, doit être rapprochée de la baisse du taux du potassium, que nous avons rapportée dans un précédent travail[2]. Etant donné ces faits, il nous semble possible d'invoquer une *compétition* dans les mouvements de l'hydrogène et du potassium, au niveau du corps ciliaire. A côté de son utilité thérapeutique, l'action de l'acétazolamide doit ainsi contribuer à préciser davantage le mécanisme de la sécrétion de l'humeur aqueuse.

Nous remercions vivement, pour leur précieuse collaboration, Melles A. M. KÖSTLIN et CH. MOSER.

A. FALBRIARD, M. C. SANZ, R. ZENDER et A. FRANCESCHETTI

Clinique Thérapeutique, clinique ophtalmologique et Laboratoire Central de l'Hôpital cantonal, Genève, le 9 juillet 1955.

Summary

In 26 rabbits, the normal pH of the aqueous humor, measured with a glass electrode, was found to be 7·59 ± 0·102. With an improved technique, the corresponding measures on ten other rabbits gave an average value of 7·57 ± 0·043.

Three hours after the injection of Diamox, a carbonic anhydrase inhibitor, a significant drop of 0·13 pH units was observed.

These effects seem to be dependent on the inhibition of the ciliary body carbonic anhydrase; however there may be some participation of the metabolic acidosis of renal origin. The fall of pH in aqueous humor produced by Diamox is to be correlated with the decrease of potassium concentration previously reported; these facts suggest the hypothesis of a competition between potassium and hydrogen ions in the secretion of aqueous humor.

[1] V. D. KINSEY, A. M. A. Arch. Ophth. *50*, 401 (1953).

[2] A. FALBRIARD, R. ZENDER, M. C. SANZ et A. FRANCESCHETTI, Exper. *11*, 232 (1955).

The Determination of Tension Wood in Ash with the Aid of the Phase-contrast Microscope

CLARKE[1], ONAKA[2], DADSWELL and WARDROP[3] have concerned themselves with investigations into the occurrence of tension wood in ash-trees (*Fraxinus* spp.).

Tension wood is a form of so-called reaction wood, occurring especially in hardwood, primarily on the upper side of limbs and stems of leaning trees.

Authors describing the occurrence of tension wood (*i.al.* RENDLE[4], JAYME and HARDERS-STEINHÄUSER[5]) assume that the most striking characteristic of tension wood visible in a cross-section is its strongly refractive layer, mostly ring-shaped, lying wholly or in part against the inner side of the fibre wall. The layer is indicated in literature among others by the following names: Gelatinous layer[4], Mucilaginous layer[6], Zugholzlamelle[5].

With the aid of staining reactions, the results of which were checked by means of ultra violet microscopy, DADSWELL and WARDROP[3] showed that no trace of lignin was present in the gelatinous wall.

Recent investigations[7] show with a considerable degree of certainty by means of X-diagrams that this exclusive layer is for the greater part composed of cellulose with a high degree of orientation.

The most characteristic method, which is consequently unanimously prescribed to demonstrate the occurrence of tension wood, is carried out in the following way. A cross-section of the wood under investigation is treated with stains or reagents eminently suitable for rendering cellulose visible.

Three methods are summarized below which are being successfully used for applying so-called contrast staining suitable for a microscopic identification of tension wood. The effect of contrast staining is due to the fact that the cellulose of the ring takes a colour different from the one taken by the wall containing lignin.

(1) *Chlor-zinc-iodine*. Chlor-zinc-iodine is the reagent most widely used for determining reaction wood. The rings are stained violet, the rest of the cell wall yellow, this effect giving a very clear contrast.

(2) *Phloroglucin-hydrochloric acid*. This reagent used to examine the lignification in the cell wall yields an uncoloured ring and a more or less reddish "wall" in reaction wood.

(3) *Safranin-fast green*. Staining with Safranin-fast green results in a green ring and a red "wall".

When, however, an attempt is made at determining the occurrence of reaction wood in ash by means of the three staining reactions afore-mentioned, the diverse results obtained in this case prove to be contradictory.

In our laboratory an investigation was carried out on wood growing at the upper side of an excentric ash branch (diameter 3 cm). After sawing, the upper side of the branch showed a glossy appearance. This feature may be closely connected with the occurrence of tension wood. Hence we may reasonably expect that there are

[1] S. H. CLARKE, Forestry *13*, 1, 68 (1939).

[2] F. ONAKA, Wood Res. Bull. Wood Res. Inst., No. 1, Kyoto University, Kyoto (1949).

[3] H. E. DADSWELL and A. B. WARDROP, *Structure, properties, and formation of tension wood.* Paper to be presented to sub-section 13b. For. Anatomy. 8th Internat. Congress Botany, Paris (1954).

[4] B. J. RENDLE, Tropical woods *52*, 11 (1937).

[5] G. JAYME and M. HARDERS-STEINHÄUSER, Das Papier *4*, 7/8, 104 (1950).

[6] E. CH. JEFFREY, *The Anatomy of woody plants* (Chicago, 1917).

[7] A. B. WARDROP and H. E. DADSWELL, Austr. J. Sci. Res. [B] *1*, No. 1, 3 (1948).

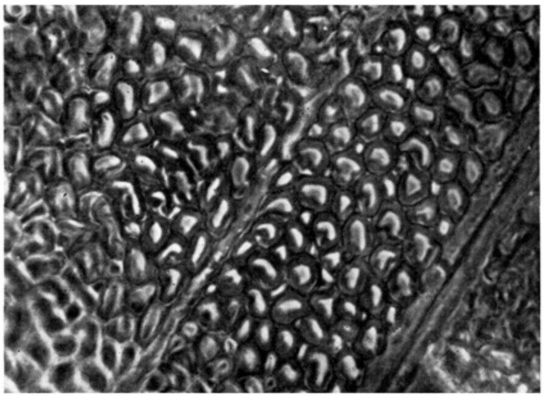

Fig. 1.–Cross-section of ash-tree containing tension wood (300 ×).

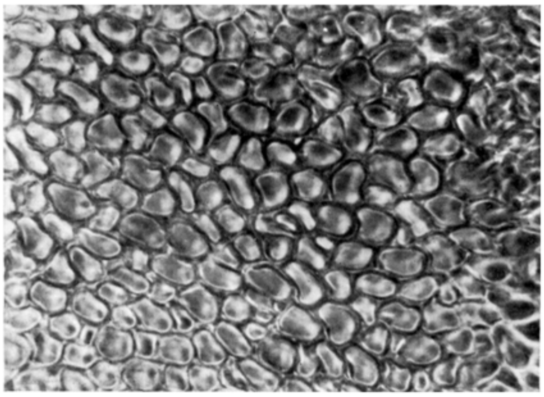

Fig. 2.–Cross-section of ash-tree containing no tension wood (300 ×).

tension wood fibres in the upper side of the branch under investigation. Cross-sections measuring 15 μ were made without pre-boiling the wood, the sections dropped into water. The results of the three staining methods applied to the cross-sections of ash wood were as follows:

(1) *Chlor-zinc-iodine.* There was no trace of violet rings. The fibre walls were stained totally yellow.

(2) *Phloroglucin-hydrochloric acid.* The entire fibre wall is stained light pink. Uncoloured rings are not observed.

(3) *Safranin-fast green.* The secondary layer stains green and the other layers red.

From these staining reactions no reliable conclusions can be drawn as to the occurrence of tension wood in ash.

The methods (1) and (2) show that no "gelatinous" ring is present and that in the cross-sections no fibres of tension wood occur.

The staining method (3) is indicative of a very thick layer of cellulose.

On account of these results, the three staining methods as such may not be used to indicate so-called tension wood. The cell wall of the tension wood fibre is likely to have a particular chemical constitution making it impossible to reveal the ring in a reliable way by means of the three staining methods. Hence, up to the present, no investigator has succeeded in determining tension wood in ash-trees[1]. Tension wood in ash remains invisible if we confine ourselves to the use of staining methods. Consequently we have made an attempt at demonstrating the occurrence of tension wood in ash quite differently, in a physical way. For this purpose we used the phase-contrast microscope.

As starting material we used cross-sections measuring 5 μ as described above and as control test material cross-sections of the wood growing at the lower side of the branch. The latter sections normally have no tension wood.

The cross-sections were examined in a suitable embedding material with the aid of the phase-contrast microscope. Figures 1 and 2 show the results, Figure 1 giving a cross-section containing tension wood. The darker ring is identical with the so-called gelatinous layer having a substance with a refractive index of about 1.536.

The use of the phase-contrast microscope actually enables us to make visible a ring in the tension wood fibre of ash-trees, it is even possible to determine the refractive index of the ring-material. Moreover, it ei obvious that the substance of the ring is not homogen-s ous, revealing a coarse grained structure, which cannot be demonstrated by staining methods or may be taken for an artefact. Figure 2 shows cross-sections containing no tension wood. The gelatinous layer is not present in this case.

The white shadow connected with the cell wall is the "halo", an artefact inherent in phase-contrast microscopy.

Conclusions. It is not possible to demonstrate visually tension wood in ash-trees by staining methods.

With the aid of the phase-contrast microscope it proves possible to reveal tension wood in the fibre wall of ash in a very simple way provided the method mentioned above is taken into account.

S. M. JUTTE and J. ISINGS

Forest Products Research Institute T.N.O. and Central Laboratory T.N.O., Delft (Netherlands), June 13, 1955.

Zusammenfassung

Es ist nicht möglich, Zugholz in Eschenholz mittels Färbungsmethoden erkennbar zu machen. Mit Hilfe des Phasenkontrast-Mikroskops kann man die Anwesenheit von Zugholz in der Faserwand von Eschenholz auf einfache Weise zeigen, wenn man die angegebene Methode genau einhält.

[1] H. E. DADSWELL and A. B. WARDROP, *Structure, properties, and formation of tension wood.* Paper to be presented to sub-section 13b. For. Anatomy. 8[th] Internat. Congress Botany, Paris (1954).

Developing Wheat Embryos Excised from Ovaries Cultured *in vitro*

UTTAMAN[1] failed in rearing proembryos of maize, and ZIEBUR and BRINK[2] did not succeed in developing barley embryos 0·3 mm or less in diameter. Ovaries of various plants (tomato, gherkin, bean, tobacco and strawberry) have been cultivated *in vitro* by NITSCH[3]. With gherkins, he found that only ovaries excised on the fourth day after pollination yielded seeds of which 6% germinated. Seeds developed in ovaries excised earlier, proved to be incapable of germinating.

[1] P. UTTAMAN, Current Sci. *18*, 215 (1949); Ref.: Ber. Wiss. Biol. *68*, 196 (1950).

[2] N. K. ZIEBUR and R. A. BRINK, Amer. J. Bot. *38*, 253 (1951).

[3] J. P. NITSCH, Amer. J. Bot. *38*, 566 (1951).