this extract for softening chitin for histological purposes, but it contains also a number of other enzymes which damage the tissues that are under examination. Moreover, puff-balls are not readily obtainable. The common mushroom of commerce also contains large amounts of chitinase, and may be obtained at any time of year. The extraction outlined below may be applied to either mushrooms or to puff-balls, and yields a liquid which will keep for a year or more in the refrigerator and is relatively free from other enzymes that might detract from its histological usefulness.

100 gm. mushrooms are roughly torn up and steeped overnight in 100 ml. 35 per cent w/v sodium chloride solution. The chitinase is soluble in this saline, while most of the other enzymes are salted out and are present in the residue which is centrifuged off. This stock solution may be kept in the refrigerator without the addition of any preservative, as the salt concentration is above the limit for bacterial or fungal growth. For use it is diluted to an appropriate salt concentration either with acetate buffer at *p*H 5 or more simply with distilled water, relying on the atmospheric carbon dioxide to produce approximately the correct *p*H. For marine organisms a dilution of 1 : 10 seems appropriate, giving a solution nearly isotonic with sea water. The fixed specimens are washed well with running tap-water overnight to remove the last traces of the fixative, which might inactivate the enzyme. They are then incubated with the diluted enzyme preparation for 12–24 hr. at 37°. A little toluene may be added to prevent bacterial action, particularly if the chitin is thick and it is desired to continue incubation for a longer period. After incubation the specimens may be prepared for sectioning in the usual way. This technique has proved successful with the copepod *Calanus*, the prawn *Palaemon* and the insect *Locusta*. It is useless with heavily sclerotinized integuments, which have a high protein content and low chitin content.

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An Improved Feed for Experimental Fish

It is well known that minced liver is an excellent food for brown trout (*S. trutta*) kept under hatchery conditions or under experimental conditions in aquaria[1]. In aquaria with a low water-flow the minced liver very quickly fouls the water. It has been found that if the liver is mixed with gelatine this difficulty can be avoided. As many workers now use these fish as experimental animals, it is felt that it would be useful to publish this fact.

The minced liver, previously salted with 5 gm. of salt per pound of liver, is mixed with 120 c.c. of a 33 per cent solution of gelatine. The whole is allowed to set and then reminced before feeding.

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Survival of Woody Plants at Extremely Low Temperatures

There are a number of things that should be pointed out in connexion with the communication by A. Sakai[1]. Various kinds of plant material have been cooled to these temperatures and even lower, yet they have survived[2–4]. My own work[5] revealed that leaves of *Pinus strobus* could be cooled to −90° C. in winter without apparent damage, and it was later shown that leaves of this same species could be cooled to −189° C. without damage as indicated by the tetrazolium test made several days after treatment[6,7]. Recently I have cooled buds of various deciduous trees to −80° C. at the rate of cooling and warming indicated in Table 1, and although some survived, others did not.

Table 1. Cold Resistance* of Tree Foliage in Deg. C. at New Haven, Connecticut, in January

Rate of cooling about 4° C. change per hr.; rate of warming about 8° C. change per hr. Most plants became still hardier in February

Plant	Origin	Resistance* ° C.
Abies guatemalensis†	Costa Rica	−6
Cupressus lusitanica†	Costa Rica	−10
Cryptomeria japonica	Japan	−20
Pinus palustris†	Northern Florida	−25
Ilex opaca	unknown	−35
Chamaecyparis pycifera	Japan	−42
Taxus baccata	unknown	−42
Tsuga canadensis	New Haven, Conn.	−45
Juniperus virginiana	Branford, Conn.	−52
Picea excelsa	Germany	−58
Tsuga canadensis	Branford, Conn.	−58
Pinus sylvestris†	Germany (?)	−62
Pinus strobus	New Haven, Conn.	> −189

* The lowest temperature which the leaves could withstand without being killed.
† Seedlings 1 to 6 years old.

I thus feel that Sakai's findings are generally in accord with my own and those of others. On the other hand, one gains the impression from Sakai's communication that if a plant can be cooled to −30° C. without damage it can be cooled on down far below this without any damage. Yet the winter resistance of several conifers can range all the way from a few degrees below freezing to below −189° C. (Table 1). But the rates of cooling and warming are most important in such work and should not be changed.

Finally, it should be understood that nearly any criterion of life or death can be at fault if not used in the correct way. Several days after the treatment is made, the tissue may die while controls survive. This error is particularly involved in plasmolysis tests which indicate the intactness of the outer vacuolar membranes but do not prove that the cytoplasm itself has not been injured. Judging by the tetrazolium test, enzymes may become less active over a period of time and finally show no activity, while untreated cells continue to reduce tetrazolium salts. The more severe the injury the more rapidly this change comes about after warming. In our experiments the time-lag effect in dying is a serious source of error in cold-treated leaves. Some leaves which survived in a moist chamber at 22° C. for two weeks died in the third week, while controls survived for at least three months.

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JENSEN[1] has also claimed that the ability to plasmolyse in onion cells is not a reliable index to their viability, because he observed some abnormal cells in which vacuoles were normally stained by neutral red solution and tonoplasts still retained their permeability, having the cytoplasmic layers torn outside the tonoplasts. In a previous report[3] I stated that such abnormal cells have not yet been observed during several years study in the parenchyma cells of the cortex in woody plants, but some parenchyma cells are found in which the ectoplasts still retain their semi-permeability though their tonoplasts are torn and their cytoplasmic layers are mixed with vacuolar content (see plate1,2 ref. 2) However, such abnormal cells are easily distinguishable from the normal by their appearance. Thus, in the parenchyma cells in twigs of woody plants, unlike the case of onion cells, it may be possible to determine the relative degree of viability in parenchyma cells, upon the basis of both their vital staining with neutral red solution and the appearance of plasmolysed cells, at least in one and the same series of experiments. However, judging[3] of the intactness of twig as a whole cannot be made only on the basis of plasmolysis test in parenchyma cells just subsequent to, or even after, many days of thawing, because inner cortex, pith ray, and pith periclinal tissue are less resistant to freezing than parenchyma cells of cortex. Accordingly, to demonstrate the intactness of a treated twig as a whole, it was planted in moist sand and its capacity tested to continue normal development at least for three months after planting, as mentioned in my reports[4]. Even in the tetrazolium[5] test used by Parker, it may be said that judging the intactness in plants means only determination of a relative value of viability of certain tissues in twig or leaf without testing the whole of it as is done by the plasmolysis method.

In my previous communication in *Nature* (and ref. 4) it was stated that almost all the easily freezable water in a cell may be drawn from the cell interior by extracellular freezing at about −30° C., and that the cells and tissues in this state are not injured even when immersed directly in liquid nitrogen, provided they can sufficiently withstand such pre-freezing at −30° C. Moreover, it was reported that below this temperature the intensity of cold seems not to exert any important effect upon woody plants, at least as long as the intense cold does not extend over a long period. Against this view, Parker has pointed out that one gains the impression from my article that if a plant can be cooled to −30° C. without damage, it can be cooled down far below this without any damage. As a result of Parker's experiment the winter resistance of several conifers could range all the way from a few degrees below freezing to below −190° C.

In the pre-freezing method, to cool plant material to −30° C. does not mean that the temperature of the material only reaches −30° C., but that its material attains at least the state of equilibrium at −30° C. and the state in which almost all the easily freezable water in a cell is drawn from the cell interior. To assure this condition, in the case of freezing in comparatively large material as a twig, I pre-froze it for 16 hr. (overnight) in all cases as mentioned in previous papers[4,5]. In the cooling method used by Parker, when a certain temperature-level was reached in the flask containing a shoot from branch, the flask was removed from the chilling apparatus, then rewarmed. In such cooling method, even if plant material is cooled to −30° C., it is doubtful whether almost all the freezable water in the cell interior can be drawn out or not within such short period of time.

The grade of frost resistance of a woody plant differs according to the manner in which it is estimated. If the grade of frost-resistance is represented by the lowest temperature at which the twigs are able to survive freezing for an hour, especially when the cooling-rate in a temperature-range below −30° C. is comparatively slow as 4 deg. C./hr., there can be found fairly great differences in the grade of frost injury in the twigs treated at an arbitrary different temperature below −30° C. This is particularly the case of less hardy species. However, when the grade of frost resistance is represented by the minimum temperature at which the twigs are able to survive freezing for a full day, there is found, in many cases, scarcely any difference in the grade of frost resistance between the material treated at −30° C. and that at −70° C. (ref. 7). It may be said, therefore, that the discrepancy between our two points of view is due mainly to the difference in the manner of representation of the grade of frost resistance. However, to clarify this problem, it seems necessary to continue further work in many woody plants.

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Pollen Germination in some Gramineae: *Pennisetum typhoideum*

POLLEN of grasses is notoriously difficult to culture, and even in Nature fertilization usually fails if pollen is not transferred directly from the anthers to the stigma[1,2]. Several previous attempts to germinate the pollen grains of the Gramineae have been unsuccessful[1-6]. In this laboratory attempts were made to germinate pollen grains of *Pennisetum typhoideum* (varieties *T*.25 and *T*.55, *I.C*.1472), *Zea mays* (*T*.41 var. Kanpur) and several varieties of *Hordeum vulgare*, *Sorghum vulgare* and *Triticum aestivum* by the hanging-drop technique (for details of technique see Vasil[7]). Best germination was obtained in *Pennisetum typhoideum*, while in the remaining plants pollen tubes longer than 600μ could not be obtained even after the addition of hormones, vitamins, mineral salts and stylar and ovarian extracts.

The pollen grains of *P. typhoideum T*.25 germinate within 10 min. of their inoculation in 10–40 per cent sucrose solutions and the optimum germination (62 per cent) occurs in 30 per cent sucrose where the tubes attain a length of 2,132μ (Fig. 1). With the addition of 0·01 per cent boric acid best growth is obtained in 25 per cent sucrose. In this medium 78 per cent of the grains germinate and the pollen tubes attain a length of 4,320μ (Fig. 1). Pollen grains of the variety *T*.55, *I.C*.1472 require lower concentrations of sugar: in 12·5 per cent sucrose there is 40 per cent germination and the tubes are 316μ long. Here also the percentage of germination is improved on the addition of 0·01 per cent boric acid, and

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