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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