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OBSERVATIONS ON VITRIFIED AND FROZEN MUSCLE FIBERS IN POLARIZED LIGHT

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That matter could exist in the vitreous state has been known for centuries, but it was not until the work of Tammann (1898), at the end of the last century, that the idea was brought forth that it should be possible to vitrify any substance. Tammann and his co-workers succeeded in vitrifying about a hundred different substances, both organic and inorganic. Those liquids which had a great tendency to sub-cool were the most easily obtained in the vitreous state. Consequently, he considered glasses as supercooled liquids, the molecular configurations in both states being the same.

It is possible, then, to represent the relationship between the states of matter and temperature diagrammatically, as shown in Fig. I. Above the boiling point, B, substances exist as gas. Between the boiling point and the freezing point, M, they are liquids. If they are cooled they become crystalline at the freezing point. However, if the cooling is sufficiently rapid they solidify in the vitreous state. When this glassy material is warmed slowly crystallization takes place within a certain range of temperatures, D.

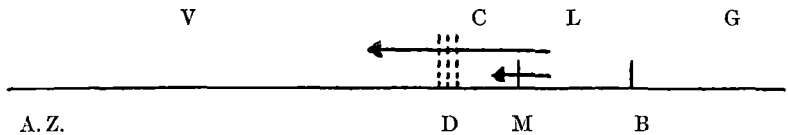

Fig. I. The relationship between the states of matter and temperature.

While the passage from the gaseous to the liquid state, or from the liquid to the crystalline state is a reversible process, the passage from the vitreous to the crystalline state is not reversible. In other words, vitreous materials may crystallize, but crystalline substances cannot be vitrified.

The crystallization zone for most substances extends only over some tens of degrees below 0° C. For a 50% gelatin gel, for example, it extends from 0° to about –12° C. If one can carry the material across these twelve degrees rapidly enough, the gel will then solidify in the vitreous state, that is, with the least possible molecular rearrangement. That this holds true for aqueous solutions and colloids in general was demonstrated by Luyet (1937). Luyet and Thoennes (1938) successfully applied this principle to protoplasmic systems. By using very thin pieces of tissue, cooling these rapidly in liquid

air, and then subsequently warming rapidly they were able to vitrify and revive the cells of the onion epidermis. Since then a number of both plant and animal tissues have been vitrified and revived in this manner.

The fundamental condition necessary for vitrification is the rapid transfer of heat in the system. By using very small particles of matter, a cooling bath of extremely low temperature, and a liquid warming bath, it is possible to insure a sufficiently rapid heat transfer to carry the material across the dangerous crystallization zone before freezing can take place.

The study of the vitrified and frozen fibers in polarized light, between crossed Nicols, was started in connection with a previously reported investigation on the properties of vitrified muscle fibers of the frog's sartorious (Thoennes, 1940), in an attempt to verify the assumption that such fibers do not contain ice.

The apparatus consisted of a horizontal polarizing microscope, the stage of which was replaced by a double-walled metal chamber 4x4x9 cm. (inside dimensions) with glass windows on two opposite sides, through which the beam of polarized light could be directed and observations made (Fig. II). The double wall provided better insulation and thus prevented too rapid changes in temperature in

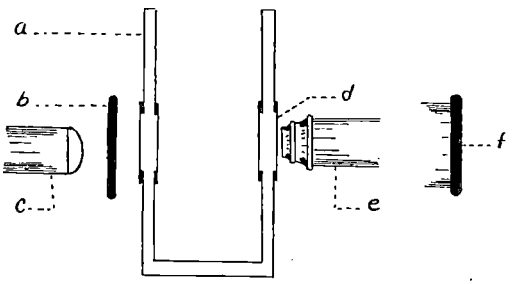

Fig. II. Diagram of apparatus used to study vitrified fibers in polarized light. (a) double-walled metal chamber, (b) polarizer, (c) light source, (d) window in chamber, (e) objective of horizontal microscope, (f) analyzer in microscopic ocular.

the inner compartment. Anhydrous calcium chloride was introduced into the space between the walls in order to absorb any excess moisture which would fog the windows at low temperatures. The inner chamber was filled with pentane, the temperature of which could be regulated by the addition of solid carbon dioxide.

Muscle fibers were teased from the frog's sartorious and placed across the prongs of a fork made of fine metal wire. The fibers adhered to the fork quite well so that it was not necessary to use any device to hold them in place. This preparation could then be suspended in the bath in such a way that the fibers were visible

through one of the windows. As the double wall of the chamber was too thick to permit the high power objective to approach close enough to the preparation, all observations were made with low power objectives. In this manner the birefringence of the entire fibers could be well observed, but variations in the individual discs were not discernable.

Observed in this manner, muscle fibers immersed in liquid air and transferred rapidly to the pentane bath at –30° C. seemed to have lost their birefringence almost completely. As the bath was allowed to warm up slowly, they began to reestablish light at about –4°C., and become completely birefringent again at 0°C.

Normal fibers, placed in pentane at 20°C., and gradually cooled to –30°C. lost some of their brightness when the temperature reached –2°C. and continued to become darker as the temperature approached –30°C. However they never became completely dark even at this last temperature.

On the other hand, muscle fibers which were completely dehydrated did not lose any of their birefringent properties when they were cooled to –30°C. in a similar manner. Later experiments gave evidence that the free water in muscle will continue to freeze until a temperature of about –56°C. is attained. This is in accordance with Brooks (1934) who found that roughly between –40°C. and –60°C. is necessary to completely freeze and free water in muscle.

When vitrified fibers were then observed in a pentane bath cooled to –60°C. they reestablished light completely. However, as the temperature of the bath rose to –56°C. they gradually became opaque, the degree of opacity increasing with further warming, until at –4°C. they again began to reestablish light. This seems to indicate that the crystallization zone for the muscle extends over approximately fifty degrees. This wide range of crystallization is no doubt an important factor in the decreased vitality of the fibers that have been vitrified and revived, as previously reported (Thoennes, 1940).

In order to study the phenomena of freezing more thoroughly, it was necessary to use higher magnifications. For this purpose a cooling chamber was devised which could be placed directly upon the stage of an ordinary polarizing microscope, the preparation being in direct contact with solid carbon dioxide. This apparatus enabled observations to be made with magnifications up to 100x.

Normal fibers were first adjusted in the position of maximal reestablishment of light. Dry ice was then applied to one end of the preparation. The fibers froze slowly and the crystals could be seen as they advanced in the fibers away from the piece of dry ice. The crystals followed all the twists and turns of the fibers (Fig. III), and the latter were seen to become practically opaque in the frozen regions. As a rule, however, the ice crystals did not extend across the entire width of the fibers, and light was reestablished in those por-

tions of the fibers not containing ice. Upon thawing the fibers become completely birefringent again.

The same method was then applied to the study of the vitrified fibers. Muscle fibers were immersed in liquid air and then transferred rapidly to the cooling chamber. The fibers failed to reestablish light due to the presence of crystals, not in the fibers as was apparently the case with the frozen muscle, but surrounding them

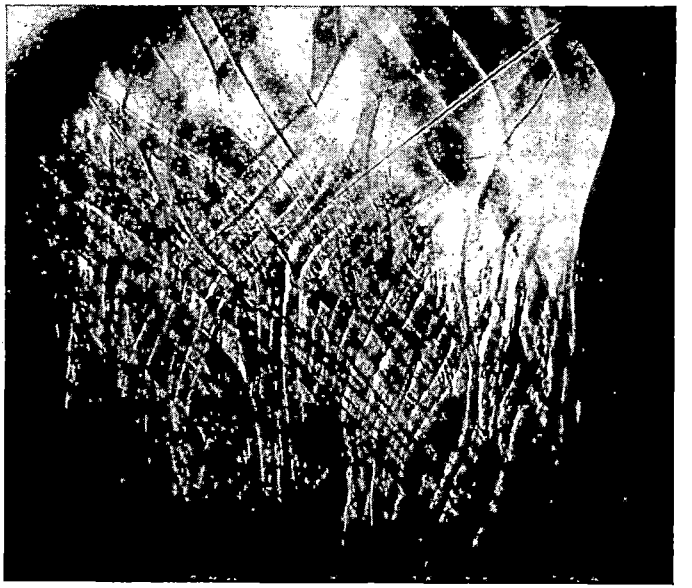

Fig. III. Ice crystals in muscle fibers.

(Fig. IV). When the excess water around the fibers was blotted off before immersion in liquid air, most of these crystals were absent, and the fibers were quite birefringent.

The question then remained as to the reason for the opacity of the crystals in the fibers. Mirsky (1937) has shown that the great mass of water in the muscle seems to be located between longitudinally arranged strips of protein in the fibers. If we accept this view, and consider that ice crystals belong to the hexagonal system, it would seem that during slow freezing, when the crystals are formed within the fibers, it is this water which crystallizes, and consequently will not reestablish light unless the fibers are viewed in cross section, when the beam of light would pass through the optical axis of the crystals.

At the present time no explanation can be offered as to the reason for the opacity of the crystals which form around the vitrified

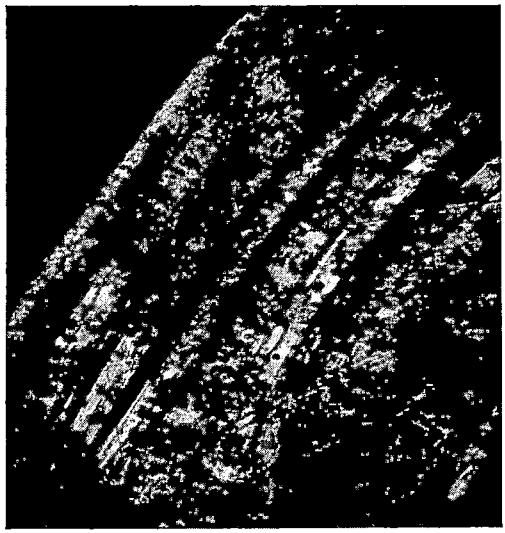

Fig. IV. Ice crystals surrounding muscle fibers after immersion in liquid air.

fibers when the excess water is not removed. It may be suggested, however, that the rate of cooling in this case may be so rapid that there is not time enough for true crystals to form, and that what is seen are simply crystalline aggregates or nuclei which disperse the light so completely as to appear dark.

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MINNESOTA WILDFLOWERS IN COLOR

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ABSTRACT

This is an attempt to stimulate appreciation of our native plants for the purpose of creating a correct wilderness attitude for preservation of natural areas. It appeals to the interest through beauty and abundance of springtime flowers. The wealth of the flora is shown by selected species in type habitats. Included is a black