Use of Trisodium Phosphate in Microscopical Technic

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The writers (*2*) published a preliminary report on the use of trisodium phosphate as a reagent for reclaiming preserved zoological specimens which have become dry by the complete evaporation of the preserving liquid. Discovery of this method was incidental to a series of studies in which various detergents were used to facilitate penetration of fluids in the preparation of microscopic mounts of parasitic worms. A series of memoranda by Hance (*1*) called attention to the fact that wetting agents added to fixing reagents hasten the penetration of the killing fluids into the tissues. Dr. Hance also pointed out that a wetting agent increases the speed with which many staining reactions are accomplished, while, added to albumin fixative, it makes the spreading of paraffin ribbons much more certain. In addition to these uses in microscopic technic, the present writers have found that the action of trisodium phosphate on preserved parasitic worms seems to increase noticeably the permeability of tissues to all the histological reagents. The effect upon the tissues seems to be much more profound than would be accounted for by the chemical merely reducing the surface tension of the fluids. Entire histologically fixed organisms become much softened when treated with trisodium phosphate, but there is no apparent damage to the tissues such as occurs when potassium hydroxide is used.

For many years in this laboratory serious difficulties have been encountered in securing permanent whole mounts of Acanthocephala. Similar difficulties are often experienced in handling nematodes and other organisms intended for microscopic study. Very commonly, stained specimens, after careful dehydration and clearing, turn chalky white and become entirely opaque after being introduced into balsam or clarite. To avoid this, it became routine procedure to prick the body wall of all specimens with a very fine needle before placing them in stain. By using a dissecting binocular, the perforations may be made in places where relatively little damage to the body wall or underlying structures results. However, in many instances internal organs are injured, and in all cases the punctures mar the appearance of the finished mounts.

It should be recalled that the entire body of all acanthocephalans is completely invested by a thin, noncellular cuticula. This plays an important role in the life processes of the worm and, even following death, continues to determine the passage of fluids through the body wall in both directions. Since there is not the slightest vestige of digestive organs at any developmental stage, the living worm must absorb all of its nutrients through its body surface. During life a very delicate balance is maintained between the fluids within the worm and those in the surrounding medium. As long as normal conditions are maintained, the body is flat and flaccid; when the living worm is removed to normal salt solution or water for microscopic examination, this balance becomes disturbed. Fluids pass through the wall, causing the body to become turgidly distended and circular in cross section. Many zoologists have failed to realize that this turgid state is due to a physiological response to abnormal surroundings and hence have classified Acanthocephala as "round" worms. Since the plumped condition is almost universally distinctive of all well-preserved specimens, it should be maintained through dehydration and clearing after the specimens have been stained and prepared for microscopic mounts.

Alcoholic specimens are passed down the grades of alcohol to water and are then placed in a solution of approximately .25 per cent of trisodium phosphate in distilled water. Freshly preserved specimens become soft, pliable, and translucent almost immediately, while specimens that have become hard, brittle, and unyielding after long preservation may require several hours or even days in a warming oven before they attain the proper degree of softness and pliability. It is well to keep treated material under observation, since the use of too strong solutions of trisodium phosphate or weaker solutions over too long a time may render specimens too soft and jelly-like for easy handling. When the desired degree of softness and translucency has been reached, they should be removed to distilled water to check the action.

Specimens thus treated, washed in distilled water, and subjected to hematoxylin or borax carmine stain become much more brilliantly stained than untreated specimens. Furthermore, the treated specimens may be dehydrated, cleared, and mounted in clarite or damar without developing opacity except in rare instances. This treatment is far superior to the puncturing mentioned previously.

Specimens of Acanthocephala which have been completely dried for many years have been reclaimed by the foregoing process. In some instances, practically all details of internal structure became available in the stained mounts; in others, only structures like proboscis hooks and body spines. With preserved specimens which are shriveled and hardened the strength of the solution is increased. Worms which are fairly turgid in preserving fluid usually become soft and flaccid in trisodium phosphate. Later, when returned to water or alcohol grades, the turgidity reappears. One distinct advantage of the softening effect is the ease with which specimens may be straightened prior to dehydration. While softened, each specimen may be folded back and forth on a microscopic slide along strips of cardboard or toothpicks and may be slightly compressed and held in place by covering with another slide. Several wrappings of thread provide the pressure and hold the slides together so that the stained specimen may be carried in Coplin or Harvard jars through the grades of alcohol. In absolute alcohol the specimen becomes firm enough that it may be removed from between the slides and yet retain its series of folds, ready for clearing and mounting under a cover glass. Such a preparation may be "read" under the microscope in much the same way that series of sections are interpreted, without the confusion of parts usually encountered when long worms are broken into lengths suitable for mounting. Large worms intended for partial dissection to show internal organs are much more readily handled after treatment with trisodium phosphate.

No other detergent tried in this laboratory has given results superior to those obtained with commercial grade of trisodium phosphate, which has the distinct advantage of low cost and general availability.

References

1. HANCE, R. F. *New histological methods.* 1938. (Mimeo.); *Gradwohl Lab. Dig.*, 1939, 2a; *Proc. Pa. Acad. Sci.*, 1940, **14**, 114–116.
2. VAN CLEAVE, H. J., and ROSS, J. A. *Science*, 1947, **105**, 318.