On a method for the silver staining of marine objects.

By

Sidney F. Harmer,

King's College, Cambridge.

The principle of this method was suggested to me by Dr. W. H. Ransom, and consists in the replacement of distilled water in the ordinary process of silver staining by a solution of a neutral salt not precipitable by silver nitrate, and of the same specific gravity as sea water. *Loxosoma* and *Pedicellina* were the first objects investigated, and these animals are not killed by an exposure of as much as half an hour to a 5 per cent solution of potassic nitrate in distilled water. It is thus quite easy to free the tissues from the greater part of their chlorides by washing with the above mentioned solution of potassic nitrate; from this the objects are transferred (naturally without the formation of any precipitate) for 4 or 5 minutes to a solution of silver nitrate ($^1/_8$—1 per cent, according to circumstances). After reduction of the silver during exposure to light in the nitrate solution, the tissues may be mounted permanently either in glycerine or in Canadabalsam. Very beautiful preparations of *Loxosoma* were easily obtained by the use of osmic acid and picrocarmine after treatment with silver nitrate. The animal may either be transferred directly from the silver solution to osmic acid ($^1/_2$ per cent) and thence to picrocarmine, reduction taking place during the process, or the osmic acid may be added after the silver has been already reduced in the potassic nitrate. In successful preparations made in the above manner, the limits of all the cells of the epidermis and of the alimentary canal are exceedingly sharply marked out, the nuclei of these cells as well as of the muscle cells, connective tissue corpuscles and other tissue elements being very distinctly stained. Besides the above mentioned forms, I have obtained good results with the epidermis of *Medusae*, *Hydroids*, *Sagitta* and *Appendicularia* (tail).

Amongst *Sponges*, Dr. G. C. J. Vosmaer has, by means of the above method, easily demonstrated an outer epithelium in *Chondrosia*,

where F. E. SCHULZE first has been unable to detect a cell layer of this kind, even by the use of silver nitrate[1]; in *Thenea*, where SOLLAS[2] has observed no cell-outlines, and in many other Sponges. Dr. ED. MEYER has successfully applied the same method to the epidermis and peritoneal epithelium of Annelids (*Tomopteris*, *Amphictenidae*), to *Teleostean* ova, etc. He has obtained good nuclear colouration by transferring the tissue after reduction in potassic nitrate to alcohol, and subsequently staining with MAYER's alcoholic carmine[3]. In *Brachiopoda*, Dr. J. F. VAN BEMMELEN has had no difficulty in using the method for the investigation of the epidermis and peritoneal epithelium.

Few animals seem to resist the action of potassic nitrate to so great an extent as *Loxosoma* and *Pedicellina*, most forms being either immediately, or after a few minutes, killed by an immersion in a 5 per cent solution of this substance. Even in many of these cases, the tissues suffer very little histological change, and can be easily stained by silver nitrate. It is possible that many other salts may be used more advantageously than potassic nitrate in washing the chlorides from the tissues without killing the animal. A $4^1/_2$ per cent solution of sodic sulphate may be used instead of the potassic nitrate, over which, however, in most cases it has no obvious advantages.

R. HERTWIG[4] has described a method for the silver staining of marine animals, consisting in treating with dilute osmic acid, washing with distilled water, until the chlorides are removed, subsequently placing in a silver nitrate solution, and reducing in distilled water. By this method, HERTWIG has been able to obtain sharply defined cell limits in the ectoderm of *Ctenophora*, but not over the whole surface of the epithelium. I have myself employed HERTWIG's method in *Loxosoma* and *Pedicellina*, but with results not nearly so satisfactory as in the method I have described, the limits of the epidermic cells being not visible over the whole surface, whilst after the employment of Dr. RANSOM's method in these two genera, I have invariably found that every epidermic cell was distinctly marked out by a sharp contour.

Naples, July 9, 1884.

---

[1] Zeitschr. f. wiss. Zool. Bd. XXIX, p. 107 and Bd. XXXI, p. 290.

[2] Ann. and Mag. of Nat. Hist. 5th Series, Vol. IX, p. 445.

[3] Mittheil. a. d. Zoolog. Stat. zu Neapel. Bd. IV, p. 521.

[4] Jenaische Zeitschr. Bd. XIV, p. 324.