

of temperature causes it to thaw and wrinkle up. In hot weather the process is in consequence not practicable except in a freezing chamber. The slices are next covered with pure glycerine, and a cover-glass placed over them, glycerine jelly is run round the edge of this, and the slide is placed in the water-oven and left till the glycerine has completely converted the gelatine into glycerine jelly. If this part of the process is delayed for some few days, the gelatine undergoes some modification by which it is rendered incapable of pectinising with the glycerine, and this impairs the value of the preparation in two ways: in the first place the refractive index of the gelatine is higher than that of the glycerine, and this interferes with the optical clearness of the preparation; and in the next place the modified gelatine exerts in process of time a bleaching action on the stained tissue, and finally entirely discharges its colour. In ignorance of these facts many of my earlier preparations were left too long before warming, and are now in consequence almost worthless.

The value of glycerine in optically despiculising a sponge-slice has already been pointed out by me,¹ and subsequently by Schulze, but frozen slices mounted in glycerine have other advantages over paraffin preparations; for one thing the tissues suffer far less contraction, indeed in this respect there is no comparison possible between the two methods; but, still more important in the study of Sponges, comparatively thick slices can be cut with better results than in the case of paraffin preparations. The value of thick slices which have suffered only a minimum of contraction is well exemplified in the case of the sterrasters of the Geodiidæ; I should never have made out the scleroblast of these spicules in paraffin-cut slices, for with a full knowledge of what to look for I have only once or twice succeeded in finding them in such preparations; while in slices obtained by the freezing process there need never be any difficulty. Similarly the only traces of scleroblasts observed in connection with the adult desmas of the Lithistida were met with in slices obtained by freezing.

Isolation of the Spicules.—In the case of the Choristida a thin fragment cut from the surface almost to the centre of the Sponge is placed on a glass slide and boiled in excess of strong nitric acid; when all the soft parts have been thus destroyed, a triangular piece of blotting paper, moistened at one corner with water, is placed with the moistened corner touching the edge of the acid on the slide; when most of the acid has been drawn off, distilled water is added to the slide from a dropping tube and drawn off by blotting paper in the same way, when as much as possible has been removed from the slide lying flat, it is raised to slope at a gentle angle and the blotting paper replaced by a dry piece, the angle is gradually increased till the slide stands vertically. A second washing with water is necessary when the fragment of Sponge operated upon is of comparatively large size; when sufficiently washed with water, absolute alcohol is added and likewise drawn off by blotting paper. The slide is placed in the water-oven and when dry a little xylol

¹ Sollas, *Ann. and Mag. Nat. Hist.*, ser. 5, vol. iv. p. 48, 1879.